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**Die Rolle des p75 Neurotrophinrezeptors bei der neuronalen Plastizität im
Hippocampus der Maus**

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Inhaltsverzeichnis

1. Einleitung	1
1.1. Hippocampus	1
1.2. Neuronale Plastizität	4
1.2.1. Adulte Neurogenese	5
1.2.2. Apoptose	6
1.2.3. Dendritische Dornen	8
1.3. Der p75 Neurotrophinrezeptor	10
2. Material und Methoden	12
2.1. Material	12
2.1.1. Versuchstiere	12
2.2. Methoden	14
2.2.1. Marker für die adulte Neurogenese im Hippocampus	14
2.2.2. Versuchsprotokolle	16
3. Ergebnisse	17
3.1. Morphologie des Hippocampus	17
3.1.1. Morphologie des Hippocampus im p75NTRExIII Knockout	17
3.2. Adulte Neurogenese im <i>Gyrus dentatus</i>	18
3.2.1. Adulte Neurogenese im p75NTRExIII Knockout	18
3.3. Apoptose	19
3.3.1. Apoptose im p75NTRExIII Knockout	20
3.3.2. Apoptose im p75NTRExIV Knockout	20
3.4. Dendritische Dornen der Körnerzellen im <i>Gyrus dentatus</i>	21
3.4.1. Dendritische Dornen im p75NTRExIII Knockout	22
3.4.2. Dendritische Dornen im p75NTRExIV Knockout	23
4. Diskussion	24
5. Zusammenfassung	30
6. Literaturverzeichnis	31
7. Anhang	38
7.1. Abbildungsverzeichnis	38
7.2. Abkürzungsverzeichnis	39
7.3. Eidesstattliche Erklärung	41
7.4. Lebenslauf	42
7.5. Liste der Veröffentlichungen	43
7.5.1. Originalarbeiten	43
7.5.2. Reviews	43
7.5.3. Kongressbeiträge	43
7.6. Danksagung	45
7.7. Beigefügte Publikationen	46

1. Einleitung

1.1. Hippocampus

Als Teil des Archipalliums liegt der Hippocampus im medialen Temporallappen des Großhirns und wird dort ventral von der Amygdala und dorsal vom *Splenum corporis callosi* begrenzt. Der Hippocampus besteht aus drei Teilen, dem *Subiculum*, dem *Cornu ammonis* und dem *Gyrus dentatus* (Krisch et al., 1987). Sein zytoarchitektonischer Aufbau ist in der Nissl-Färbung dreischichtig und unterscheidet sich somit deutlich vom sechsschichtigen Aufbau der Großhirnrinde (Krisch et al., 1987). Die äußere Schicht des Hippocampus ist zellarm und wird als *Stratum moleculare* bezeichnet. Die mittlere Schicht enthält im *Cornu ammonis* und im *Subiculum* Pyramidenzellen und wird *Stratum pyramidale* genannt. Im *Gyrus dentatus* enthält diese Schicht Körnerzellen und wird demzufolge *Stratum granulosum* genannt. Die dritte Schicht des Hippocampus wird im *Subiculum* *Stratum multiforme*, im *Cornu ammonis* *Stratum oriens* und im *Gyrus dentatus* *Hilus fasciae dentatae* genannt (Krisch et al., 1987). Bei genauerer Betrachtung zeigt sich, dass das *Stratum moleculare* im *Cornu ammonis* noch weiter unterteilt werden kann (Abbildung 1). Man unterscheidet hier das *Stratum lucidum*, das *Stratum radiatum* und das *Stratum lacunosum-moleculare* (Boccara et al., 2015).

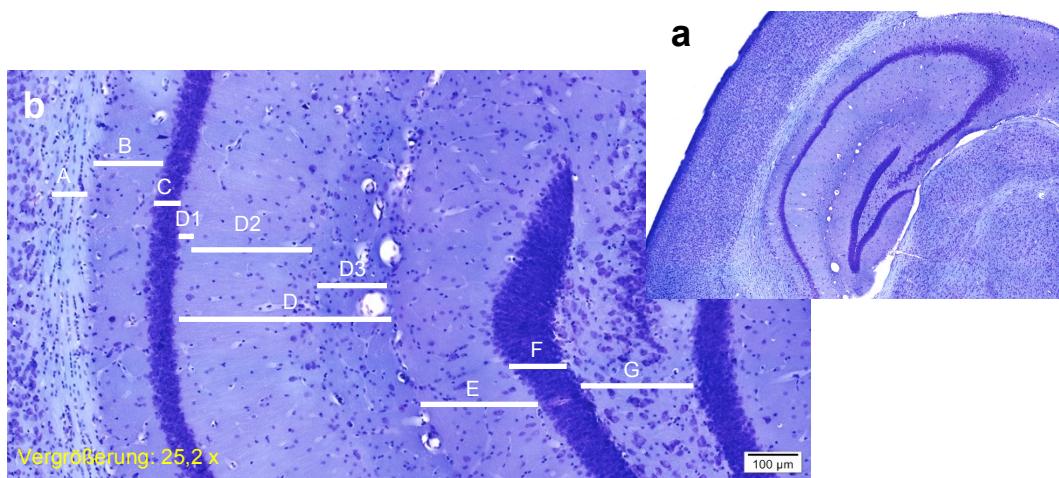


Abbildung 1: Schichten des Hippocampus in der Nissl-Färbung.

a) Übersicht Hippocampus, Koronarschnitt 30 µm, 12,6 x. b) Ausschnitt aus dem Hippocampus einer männlichen Wildtyp-Maus, 25,2 x. A Alveus, B Stratum oriens, C Stratum pyramidale, D Stratum moleculare, D1 Stratum lucidum, D2 Stratum radiatum, D3 Stratum lacunosum-moleculare, E Stratum moleculare, F Stratum granulosum, G Hilus fasciae dentatae.

Zwischen dem *Cornu ammonis* und der *Regio entorhinalis* liegt das *Subiculum*, welches eine Übergangsregion zur *Regio entorhinalis* darstellt. Die *Regio entorhinalis* stellt die Haupteingangsquelle für kortikale Afferenzen zum Hippocampus dar (Abbildung 2). Ihrseits erhält die *Regio entorhinalis* Afferenzen aus dem perirhinalen und parahippocampalen Kortex. Der perirhinale Kortex erhält vielfältige Afferenzen, beispielhaft genannt sei hier der *Bulbus olfactorius*, die *Insula*, der *Gyrus cinguli* und der *Gyrus temporalis superficialis* (Squire und Zola, 1996).

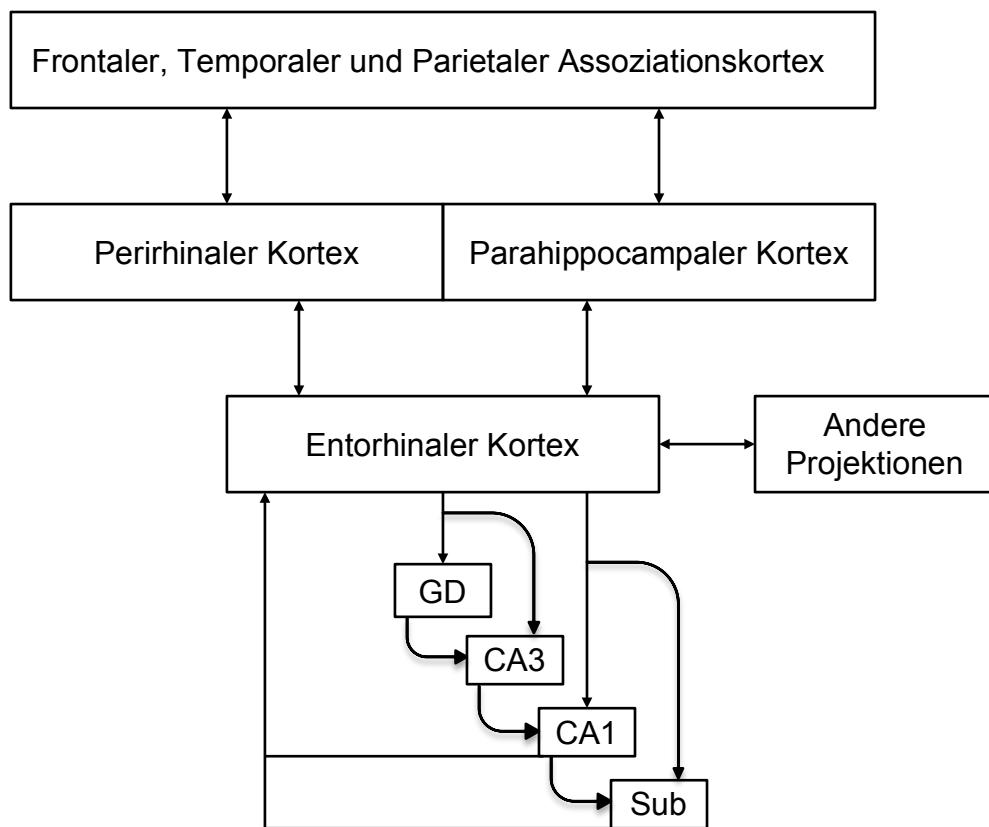


Abbildung 2: Schematische Übersicht der Verbindungen innerhalb des medialen Temporallappens.

Der entorhinale Kortex ist die Hauptquelle kortikaler Afferenzen zum Hippocampus (GD = *Gyrus dentatus*, CA1 und CA3 = Teilstufen des *Cornu ammonis*, Sub = *Subiculum*). Er erhält seine Afferenzen aus frontalen, temporalen und parietalen Assoziationsarealen über den perirhinalen und parahippocampalen Kortex (modifiziert nach: (Squire und Zola, 1996)).

Zur Beschreibung der Informationsverarbeitung im Hippocampus stellt der sogenannte Trisynaptische Kreis (Abbildung 3) ein anerkanntes Schema dar. Der Trisynaptische Kreis hat seinen Ursprung in der *Regio entorhinalis*. Von dort projizieren Neuronen im *Tractus perforans* zum *Gyrus dentatus* und bilden mit ihren *Synapsen en passant* Verbindungen mit den dendritischen Dornen der

Axone der Körnerzellen. Die Körnerzellen wiederum senden ihre Axone, Moosfasern genannt, zu den Pyramidenzellen der CA3-Region. Die Axone der CA3-Pyramidenzellen ihrerseits verlassen den Hippocampus über die *Fimbria hippocampi*, geben aber zuvor Kollateralen ab. Diese als Schaffer-Kollateralen bezeichnete Verbindung projiziert in die CA1-Region. Die Axone der CA1-Pyramidenzellen verlassen den Hippocampus über den *Alveus* und schließen sich der Projektion der CA3-Region an. Gemeinsam erreichen sie das Septum, den Hypothalamus und den kontralateralen Hippocampus (Anderson et al., 1971, Naber et al., 2000).

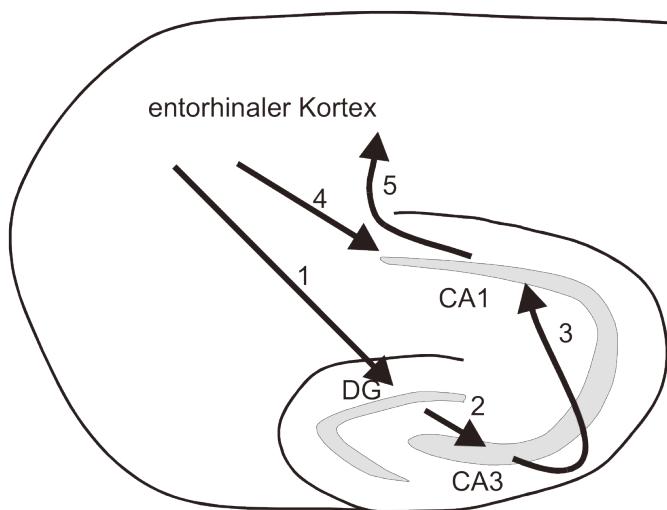


Abbildung 3: Der trisynaptische Kreis; Synaptische Verbindungen in der Hippocampusformation.

- (1) Tractus perforans (Regio entorhinalis -> Gyrus dentatus)
- (2) Moosfasern (Gyrus dentatus -> CA3)
- (3) Schaffer-Kollateralen (CA3 -> CA1)
- (4) Ein Teil des Tractus perforans projiziert in die CA1-Region
- (5) Die Neuronen der CA1-Region projizieren in Richtung Septum und Hypothalamus, aber auch zurück in die Regio entorhinalis.

(modifiziert nach: (Hasselmo und McClelland, 1999))

Der Hippocampus spielt eine zentrale Rolle beim Lernen und der Ausbildung des Gedächtnisses. In den Jahren 1954 und 1957 veröffentlichte W.B. Scoville den Fall des Patienten H.M., bei dem es nach beidseitiger temporaler Lobektomie, unter Einschluss großer Teile des Hippocampus, zu einer schweren anterograden Amnesie gekommen war. Diese Störung betraf das deklarative, das episodische und das räumliche Gedächtnis (Scoville, 1954, Scoville und Milner, 1957).

Neuere Studien mithilfe der Magnetresonanztomografie deuten darauf hin, dass eine Verringerung des Volumens des Hippocampus mit der Pathophysiologie der Depression verbunden ist (Eker und Gonul, 2010). Ferner konnte beobachtet werden, dass eine medikamentöse antidepressive Therapie bei depressiven Patienten in der Lage ist, eine Atrophie des Hippocampus zu stoppen oder sogar rückgängig zu machen (Schmidt und Duman, 2007). Interessanterweise führte eine chronische medikamentöse antidepressive Therapie im Tiermodell nicht nur zu einer Reduktion der depressiven Symptome, sondern auch zu einer Steigerung der Zellproliferation, insbesondere der adulten Neurogenese im Hippocampus (Chen et al., 2006, Blugeot et al., 2011). Weiterhin ist bekannt, dass der Hippocampus eine Hirnregion darstellt, welche einer ständigen Reorganisation unterworfen ist. So werden dort existierende neuronale Verschaltungen umweltabhängig auf der Ebene der dendritischen Dornen und der adulten Neurogenese moduliert (von Bohlen und Halbach, 2009). Diese ständige Reorganisation ist ein Ausdruck der neuronalen Plastizität des Gehirns.

1.2. Neuronale Plastizität

Unter neuronaler Plastizität versteht man die Fähigkeit des Gehirns sich sowohl physiologischen als auch umweltbedingten Veränderungen anzupassen. Diese Fähigkeit besteht von der Entwicklung bis ins hohe Alter. Zu den Prozessen, die auf zellulärer Ebene Grundlage dieser Plastizität sind, zählen einerseits die adulte Neurogenese, also die Entstehung und Differenzierung von Neuronen aus neuronalen Stammzellen, und andererseits Veränderungen in der Morphologie und Aktivität von differenzierten Neuronen (Mainardi et al., 2015). Hier sei beispielhaft auf die dendritischen Dornen verwiesen, welche im Hippocampus die Hauptquelle des exzitatorischen Inputs darstellen und ständigen Änderungen in Form, Länge und Dichte unterlegen sind (von Bohlen und Halbach, 2009).

1.2.1. Adulte Neurogenese

Für eine lange Zeit wurde angenommen, dass adulte Neurogenese ein Phänomen ist, welches ausnahmslos während der Zeit der Gehirnentwicklung auftritt. Die revolutionären Arbeiten von Joseph Altman und Gopal Das zeigten jedoch, dass Neurogenese auch postnatal auftritt und somit nicht ausschließlich als Entwicklungsphänomen zu betrachten ist. In einer ersten Studie 1965 konnten sie mit Hilfe der Autoradiografie-Methode zeigen, dass im *Gyrus dentatus* von Ratten postnatal Neurogenese stattfindet (Altman und Das, 1965). Wenige Jahre später zeigten sie mit der gleichen Methode die postnatale Entstehung von Neuronen auch in Meerschweinchen (Altman und Das, 1967). Die ersten Hinweise auf eine funktionelle Bedeutung der adulten Neurogenese lieferten die Befunde aus der Arbeitsgruppe um Fernando Nottebohm. In ihren Untersuchungen des Hirnkerns *Hyperstriatum ventrale Pars caudalis* (HVC) von Kanarienvögeln, einer Hirnregion, welche in die Stimmkontrolle involviert ist, konnten sie zeigen, dass dort adulte Neurogenese stattfindet (Goldman und Nottebohm, 1983). Diese neu entstandenen Neuronen wurden sogar in bestehende neuronale Verschaltungen integriert und somit funktional relevant (Paton und Nottebohm, 1984). Weiterhin konnten sie zeigen, dass es in dieser Region erstaunlicherweise zu keiner Zunahme der Gesamtzellzahl kommt, sondern dass vielmehr ein *turnover*, also ein ständiges Absterben und Neuentstehen von Neuronen vorliegt. Dieser *turnover* scheint die Grundlage der Stimm- und Gesangskontrolle zu sein und ist somit mit Lernvorgängen assoziiert (Nottebohm, 1985).

Die Etablierung neuer Methoden, insbesondere die Injektion und Detektion von Bromodesoxyuridin (BrdU), ermöglichen die weitere Erforschung der adulten Neurogenese. So konnte gezeigt werden, dass die adulte Neurogenese im Hippocampus an der Grenze zwischen *Hilus* und *Stratum granulosum* stattfindet (Kuhn et al., 1996). Interessanterweise ist die adulte Neurogenese ein Prozess, der sich physiologischen, aber auch umweltbedingten Veränderungen anpasst. Denn sowohl hirnelektrische Phänomene auslösende Versuche, wie die Pilocarpin-induzierte Epilepsie (Parent et al., 1997) und das sogenannte *Kindling* (Scott et al., 1998), als auch eine angereicherte Umwelt

(*enriched environment*) führen zu einer Steigerung der Neurogeneserate (Kempermann et al., 1997). Diese Befunde stammten jedoch ausnahmslos aus der Untersuchung von Nagern. Bereits an dieser Stelle sei angemerkt, dass es für das Verständnis von Änderungen in der Neurogeneserate entscheidend ist, Kontrolle der Neurogenese und Regulation der Neurogenese zu unterscheiden. Während Kontrolle die Unterhaltung einer *Baseline* der Neurogenese beschreibt, ist mit Regulation eher die Abweichung der Neurogeneserate von dieser *Baseline* gemeint (Kempermann, 2011).

Erst 1998 gelang es der Arbeitsgruppe um Fred Gage zu zeigen, dass auch im menschlichen Hippocampus postnatal Neurogenese stattfindet. In Sektionsmaterial von Tumorpatienten, die BrdU zu diagnostischen Zwecken erhalten hatten, konnte in Hirnproben des Hippocampus die Entstehung neuer Neuronen gezeigt werden (Eriksson et al., 1998).

Wie schon von Nottebohm und Kollegen für Singvögel beschrieben, konnte auch für Nager gezeigt werden, dass es keine Zunahme der Zahl der Körnerzellen im *Gyrus dentatus* mit dem Alter gibt, wie es bei lebenslanger Neurogenese anzunehmen wäre (von Bohlen und Halbach und Unsicker, 2002). Demnach scheint auch im Hippocampus nicht die bloße Zunahme der Zellzahl wichtig für dessen Funktion zu sein, sondern ebenfalls die Rate des *turnovers*, welche als Balance zwischen Neurogenese und Apoptose anzusehen ist (von Bohlen und Halbach, 2010).

1.2.2. Apoptose

Vom altgriechischen Wort ἀποπίπτειν (apopitein) stammend, bedeutet Apoptose 'abfallen'. Apoptose als Form des programmierten Zelltods ist für die normale Entwicklung des Nervensystems von großer Bedeutung. Denn dafür ist das exakte Zusammenspiel von Zellentstehung und Zelltod, insbesondere das Gleichgewicht der Anzahl entstehender Neuronen in Relation zu den postsynaptischen Zielzellen, zwingend erforderlich (Roth und D'Sa, 2001).

Von besonderer Bedeutung für den intrazellulären Ablauf der Apoptose ist eine Familie von Proteasen, welche in ihrem aktiven Zentrum einen Cysteinrest haben und ihre Zielproteine an Aspartatresten schneiden. Sie werden deshalb Caspasen (C für Cystein und asp für Aspartat) genannt. Intrazellulär liegen Caspasen als inaktive Procaspasen vor. Ihre Aktivierung erfolgt mittels proteolytischen Schneidens durch bereits aktivierte Caspasen (Alberts et al., 2014). Es werden zwei Klassen von Caspasen unterschieden:

- A) Initiatorcaspasen: sie katalysieren die Aktivierung weiter *downstream* gelegener Caspasen und von Effektorcaspasen. Zu den Initiatorcaspasen zählen die Caspasen 2, 8, 9, 10.
- B) Effektorcaspasen: hierzu zählen die Caspasen 3, 6, 7. Sie sind in der Lage, einerseits weitere Effektorcaspasen zu aktivieren und andererseits die eigentliche proteolytische Zerstörung der Zelle in Gang zu setzen. Zu den Zielproteinen der Effektorcaspasen zählen die nukleären Laminine, das Cytoskelett, Inhibitoren der Endonukleasen und Zell-Zell-Adhäsionsproteine (Alberts et al., 2014).

Zwei Mechanismen zur Auslösung der Apoptose sind bekannt. Der sogenannte extrinsische Weg führt über eine Aktivierung von bestimmten Zelloberflächenrezeptoren zu einer Aktivierung gewisser Caspasen. Ein bekanntes und weitgehend beschriebenes Beispiel hierfür ist die Aktivierung des Fas-Rezeptors durch den Fas-Liganden. Hierbei kommt es zur Bildung eines als *death-inducing-signaling-complex* (DISC) durch Rekrutierung und Aktivierung intrazellulärer Adapterproteine an die cytosolische Todesdomäne des Rezeptors und dadurch wiederum zur Rekrutierung und Aktivierung der Procaspasen 8 und 10. Einmal aktiviert, können diese dann ihrerseits weitere Procaspasen aktivieren. Der sogenannte intrinsische Weg der Apoptose ist eng an die Integrität der mitochondrialen Membran gebunden. Als wesentliches Signalmolekül fungiert hier Cytochrom C, welches normalerweise Bestandteil der Atmungskette ist. Gelangt dies bei Störungen der mitochondrialen Membran in das Cytosol der Zelle bewirkt es die Oligomerisierung des *apoptotic protease activating factor-1* (Apaf-1) zum sogenannten Apoptosom. Im Apoptosom werden mehrere Procaspasen 9 in räumliche Nähe gebracht, so dass sie in der Lage sind, sich gegenseitig zu aktivieren (Alberts et al., 2014).

Eine Gruppe von Proteinen der Bcl2-Familie ist maßgeblich an der Regulation der Integrität der mitochondrialen Membran beteiligt. Innerhalb dieser Familie werden drei Gruppen von Proteinen unterschieden. Es handelt sich dabei einerseits um pro-apoptotische BH123-Proteine (z.B. Bax, Bak) und BH3-only Proteine (z.B. Bad, Bim, Bid) und andererseits um anti-apoptotische Bcl2-Proteine. Ihr Verhältnis zueinander ist entscheidend an der Aufrechterhaltung oder dem Verlust der Integrität der mitochondrialen Membran beteiligt. Während pro-apoptotische Stimuli die Aggregation von BH123-Proteinen in der äußeren Mitochondrienmembran fördern und so zur Freisetzung von Cytochrom C führen, hemmen die anti-apoptotischen Bcl2-Proteine dies, indem sie mit den BH123-Proteinen interagieren. Die Vertreter der Gruppe der BH3-only-Proteine stellen die größte Gruppe innerhalb der Bcl2-Familie dar und sind als wichtige Verbindung zwischen externen Stimuli und dem intrinsischen Weg anzusehen. So kann es dazu kommen, dass die Synthese oder Aktivität bestimmter BH3-only-Proteine geblockt und so die Apoptose verhindert wird, oder es kommt zu einer verstärkten Produktion von BH3-only-Proteinen mit dann pro-apoptotischer Wirkung. Weiterhin wird durch Mitglieder der BH3-only-Proteine eine Verbindung zwischen dem extrinsischen und dem intrinsischen Weg hergestellt (Alberts et al., 2014).

1.2.3. Dendritische Dornen

Die Dendriten der Projektionsneuronen in den meisten Hirnregionen sind übersät mit einer Vielzahl kleiner Aufreibungen, bekannt als dendritische Dornen. Als subzelluläre Kompartimente sind sie wesentlich an dem Empfang und der Verarbeitung synaptischer Informationen beteiligt (Whitford et al., 2002). Für den Hippocampus konnte gezeigt werden, dass sie die Hauptquelle des exzitatorischen Inputs an den Pyramidenzellen der CA1-Region (Megias et al., 2001) sind und dort zumeist nur eine einzige synaptische Bindung eingehen (Andersen, 1990). Auf morphologischer Ebene wird ein dendritischer Dorn eingeteilt in einen Kopf, welcher über den Hals mit dem Schaft verbunden ist. Je nach Ausprägung der verschiedenen Teile unterscheidet man *Mushroom*, *Thin*, *Stubby* und *Filopodia* Formen (Peters und Kaiserman-Abramof, 1970). Diese Klassifikation ermöglicht eine Beschreibung der dynamischen

Einleitung

Veränderungen in der Morphologie der dendritischen Dornen, welche jedoch lediglich als Momentaufnahme dieses Prozesses zu betrachten ist (Parnass et al., 2000).

Untersuchungen zur Ultrastruktur der dendritischen Dornen haben gezeigt, dass Neurotransmitterrezeptoren fast ausschließlich im Kopf der dendritischen Dornen, einer Region die postsynaptischen Densität (PSD) genannt wird, liegen (Nimchinsky et al., 2002). Die postsynaptische Densität ist ein elektronenmikroskopisches Korrelat für eine Region in der Rezeptoren, Adapterproteine, Zytoskelettbestandteile und intrazelluläre Signalmoleküle räumlich eng zusammenliegen und die funktionell als Schaltstelle der synaptischen Verarbeitung betrachtet werden kann. Weiterhin ist der sogenannte *spine apparatus* von entscheidender Bedeutung bei der Verarbeitung des synaptischen Inputs, stellt er doch einen Teil des glatten endoplasmatischen Retikulums und somit den intrazellulären Kalziumspeicher dar (von Bohlen und Halbach, 2009).

Die Dichte der dendritischen Dornen ist ein Maß für die Konnektivität der entsprechenden Zelle. Veränderungen in der Dichte der dendritischen Dornen werden unter anderem mit Lern- und Gedächtnisvorgängen in Zusammenhang gebracht. Bereits 1994 konnte gezeigt werden, dass die Dichte der dendritischen Dornen an den CA1 Pyramidenzellen von Ratten um 9 % zunahm, nachdem diese Tiere ein räumliches Training absolviert hatten. Ferner stieg dadurch auch ihre Fähigkeit zum räumlichen Lernen, zum Beispiel im sogenannten *Morris water maze* – Versuch (Moser et al., 1994).

1.3. Der p75 Neurotrophinrezeptor

Der p75 Neurotrophinrezeptor (p75NTR) bindet alle Neurotrophine (*brain-derived neurotrophic factor* (BDNF), *nerve growth factor* (NGF), *neurotrophine 3* (NT3) und *neurotrophine 4* (NT4)) sowie ihre jeweiligen Vorstufen (Underwood und Coulson, 2008), jedoch mit geringerer Affinität (Hu et al., 2002). Der Name p75 stammt vom Molekulargewicht dieses Proteins von 75 kD (Chen et al., 2009). Als Mitglied der Tumornekrosefaktor-Rezeptor-Familie ist der p75NTR ein Transmembranprotein und besteht aus einer extrazellulären Domäne, einer Transmembrandomäne und aus einer intrazellulären Domäne. Die extrazelluläre Domäne besteht aus vier Cystein-reichen Wiederholungen, von denen die dritte und vierte für die Neurotrophinbindestelle verantwortlich sind. Die intrazelluläre Domäne wird wie bei den Tumornekrosefaktor-Rezeptoren als Todesdomäne bezeichnet. Sie unterscheidet sich jedoch von diesen, was sich unter anderem darin äußert, dass sich p75NTRen in Lösung nicht assoziieren (Chen et al., 2009).

Interessanterweise scheint der p75NTR, obwohl er zur Familie Tumornekrosefaktor-Rezeptoren gehört und über eine Todesdomäne verfügt, primär nicht den extrinsischen Weg der Apoptose auszulösen. Vielmehr deuten die bisher publizierten Ergebnisse darauf hin, dass der p75NTR seine pro-apoptotischen Signale über die Aktivierung des intrinsischen Wegs vermittelt (Troy et al., 2002).

Es ist darüber hinaus bekannt, dass durch alternatives Splicing eine verkürzte Isoform des p75NTR entsteht; diese wird s-p75NTR genannt. Auf molekularer Ebene unterscheiden sich diese beiden Isoformen lediglich darin, dass dem s-p75NTR drei der vier Cystein-reichen Wiederholungen fehlen und er somit nicht in der Lage ist Neurotrophine zu binden (von Schack et al., 2001, Dechant und Barde, 2002). Die Funktionen dieser verkürzten Isoform sind weitestgehend unbekannt. Es wird jedoch vermutet, dass es sich trotz der fehlenden Neurotrophinbindestelle um einen funktionellen Rezeptor handelt (Fujii und Kunugi, 2009).

Die Expression des p75NTR erreicht ihren Höhepunkt während der Entwicklung des Nervensystems. Jedoch führen verschiedene pathologische Zustände wie

fokale Ischämie, mechanische Schäden, Axotomie und Epilepsie zu einem Anstieg der Expression (Dechant und Barde, 2002). Im adulten Gehirn wird der p75 Rezeptor unter anderem im Hippocampus exprimiert (Barrett et al., 2005). Auch außerhalb des Nervensystems findet sich die Expression des p75NTR. Hier konnte gezeigt werden, dass dieser in Hodengewebe, Thymus, Dünndarm, Milz und in geringerem Ausmaß auch in Herz, Muskel, Lunge und Leber exprimiert wird (Lomen-Hoerth und Shooter, 1995). Dies legt den Schluss nahe, dass Neurotrophine auch außerhalb des Nervensystems von Bedeutung sind.

In verschiedenen Studien konnte in den vergangenen Jahren gezeigt werden, dass der p75NTR einen Einfluss auf die Morphologie des Hippocampus, die cholinerge Innervation, die adulte Neurogenese, die Spinogenese und bestimmtes Hippocampus-assoziiertes Verhalten hat (Naumann et al., 2002, Zagrebelsky et al., 2005, Catts et al., 2008, Barrett et al., 2010, Bernabeu und Longo, 2010, Colditz et al., 2010, Greferath et al., 2012). Auffällig ist hier, dass diese Ergebnisse zum Teil Widersprüche aufweisen (Dokter et al., 2015, Poser et al., 2015). Aufgrund dessen sollen mit dieser Arbeit die Auswirkungen des Fehlens des p75NTR auf die Morphologie des Hippocampus, die adulte Neurogenese und die Spinogenese reevaluiert und für den s-p75NTR erstmalig untersucht werden.

2. Material und Methoden

2.1. Material

2.1.1. Versuchstiere

Für die durchgeführten Versuche standen zwei Mauslinien zur Verfügung. Angesichts der bereits publizierten und zum Teil in Konflikt stehenden Ergebnisse soll hier angemerkt werden, dass es von entscheidender Bedeutung ist, bei der Untersuchung von Knockout-Tieren stets Kontrolltiere des gleichen genetischen Hintergrunds zu verwenden (Naumann et al., 2002).

Innerhalb des p75NTR stellen vier Cystein-reiche Wiederholungen ein wesentliches Element der Ligandenbindungsstelle des p75NTR dar. Diese Cystein-reichen Wiederholungen werden vom Exon 2 und 3 des p75NTR-Gens codiert. Bereits 1992 gelang es, das Exon 3 des p75NTR-Gens zu entfernen und so eine Mauslinie ohne funktionellen p75NTR ($p75\text{NTRE}_{\text{ExIII}}^{-/-}$) zu generieren (Lee et al., 1992) (Abbildung 4). Wir erhielten diesen Mausstamm von den *Charles River Laboratories* in ihrem originalen genetischen Hintergrund ($\text{B6.129S4-Ngfr}^{\text{tm}1\text{Jae}}/\text{J}$). Homozygote Knockout-Tiere ($p75\text{NTRE}_{\text{ExIII}}^{-/-}$) wurden stets mit altersentsprechenden Tieren ($p75\text{NTRE}_{\text{ExIII}}^{+/+}$) des gleichen genetischen Hintergrunds verglichen. Diese Tiere erhielten wir durch die Kreuzung von heterozygoten $p75\text{NTRE}_{\text{ExIII}}$ Tieren.

Bedingt durch die Existenz einer alternativen Splicevariante des Exon 3 im p75NTR-Gen bleibt in den Tieren des $p75\text{NTRE}_{\text{ExIII}}$ -Stammes eine intakte Variante des p75NTR zurück, welche jedoch keine Bindung mit Neurotrophinen eingehen kann. In der neu generierten Knockoutlinie war das Exon 4 Ziel der genetischen Veränderung. Die so entstandene Knockoutlinie wurde $p75\text{NTRE}_{\text{ExIV}}$ genannt und exprimiert keine der beiden Splicevarianten des p75NTR (Abbildung 4). Diese Tiere basieren auf dem genetischen Hintergrund der Mauslinie C57BL6. Wieder wurden homozygote Knockout-Tiere ($p75\text{NTRE}_{\text{ExIV}}^{-/-}$) stets im Vergleich mit altersentsprechenden Tieren ($p75\text{NTRE}_{\text{ExIV}}^{+/+}$) des gleichen genetischen Hintergrunds analysiert.

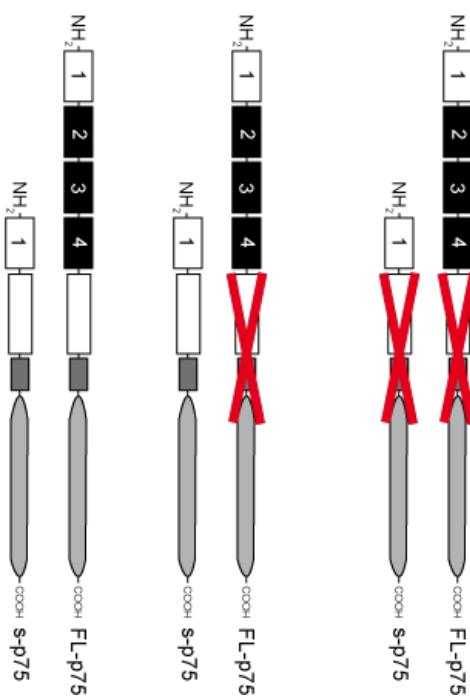


Abbildung 4: Der p75 Rezeptor in den verfügbaren Mauslinien.

s-p75 = durch alternatives Splicen entstandene Isoform des p75NTR ohne Neurotrophinbindestelle. FL-p75 = p75NTR mit Neurotrophinbindestelle. Links: Wildtyp, Mitte: p75NTRExIII, Rechts: p75NTRExIV. Die roten Kreuze markieren die jeweils nicht exprimierten Aminosäuresequenzen (nach (von Schack et al., 2001)).

Da seit langem bekannt ist, dass der Hippocampus sowohl in Nagern und Affen als auch in Menschen starken altersabhängigen Änderungen in Funktion und Morphologie unterworfen ist (Greene und Naranjo, 1987, Walker et al., 1988, Lee et al., 1994, Rapp und Heindel, 1994, Rapp und Gallagher, 1996, Driscoll et al., 2003), ließen wir einen Teil der Mäuse altern, um so eventuelle p75NTR bedingte Veränderungen zu analysieren. Für unsere Untersuchungen verwendeten wir einerseits adulte Tiere (4 – 6 Monate) und andererseits gealterte Tiere (mindestens 20 Monate).

2.2. Methoden

2.2.1. Marker für die adulte Neurogenese im Hippocampus

Adulte Neurogenese im Hippocampus findet in der Subgranularschicht des *Gyrus dentatus* statt und besteht aus fünf Entwicklungsstadien (Proliferation, Differenzierung, Migration, Zielfindung, Synaptische Integration), welche durch bestimmte morphologisch unterscheidbare Zelltypen (Typ-1-Zelle, Typ-2-Zelle, Typ-3-Zelle) charakterisiert werden (von Bohlen und Halbach, 2011) (Abbildung 5). Bei den Typ-1-Zellen handelt es sich um dreieckig geformte, sich selten teilende und morphologisch Gliazellen ähnelnde Zellen, welche die Intermediärfilamente *glial fibrillary acidic protein* (GFAP) und Nestin exprimieren. Aus diesen Zellen entstehen sich schnell teilende Vorläuferzellen, die sogenannten Typ-2-Zellen. Sie haben einen schmalen Zellkörper, einen unregelmäßig geformten Zellkern und kurze horizontal orientierte Fortsätze. Zellen dieses Typs exprimieren sowohl einige gliale als auch einige neuronale Marker. Basierend auf der Expression von Nestin, lassen sich zwei Nestin-positive Subpopulationen beschreiben, von denen eine positiv und eine negativ für den Marker junger neuronaler Zellen Doublecortin ist (Typ 2 a und Typ 2 b). Zellen des Typ 3 befinden sich in einer Übergangsphase hin zu postmitotischen Zellen, welche sich unter normalen Bedingungen nicht mehr teilen. Sie exprimieren in dieser Entwicklungsstufe lediglich neuronale Marker. Die Morphologie dieser Zellen hängt von ihrem Entwicklungsfortschritt ab. Nach kurzer Wanderung in das *Stratum granulosum* verändert sich die Ausrichtung ihrer Fortsätze von horizontal nach vertikal und auch die Komplexität der Fortsätze nimmt zu (im Übersichtsartikel (Dokter und von Bohlen und Halbach, 2012) zusammenfassend dargestellt).

Zur Beschreibung der Rate der adulten Neurogenese ist es einerseits wichtig, eine Aussage über die Rate der Proliferation zu treffen und andererseits zu untersuchen, wie viele Zellen Marker für neu entstandene Neuronen exprimieren. Als Proliferationsmarker verwendeten wir das phosphorylierte Histon 3. Es gehört zum Histon-Oktamer und liegt in dieser Form in der G2- und in der M-Phase des Zellzyklus vor (von Bohlen und Halbach, 2011). Zu

beachten ist, dass mit der Verwendung dieses Markers lediglich eine Aussage bezüglich der Zellproliferation getroffen werden kann. Die Unterscheidung in eine gliale und eine neuronale Linie ist hiermit nicht möglich (Dokter und von Bohlen und Halbach, 2012). Erst durch die Verwendung eines Markers für neu entstandene Neuronen, können neu entstandene Gliazellen sicher von Neuronen unterschieden werden. Hierzu verwendeten wir den Marker Doublecortin. Dabei handelt es sich um ein Mikrotubuli-assoziiertes Protein, welches von migrierenden und zielsuchenden Neuronen exprimiert wird. Die Expression von Doublecortin endet, wenn das neu entstandene Neuron synaptisch integriert ist (von Bohlen und Halbach, 2007).

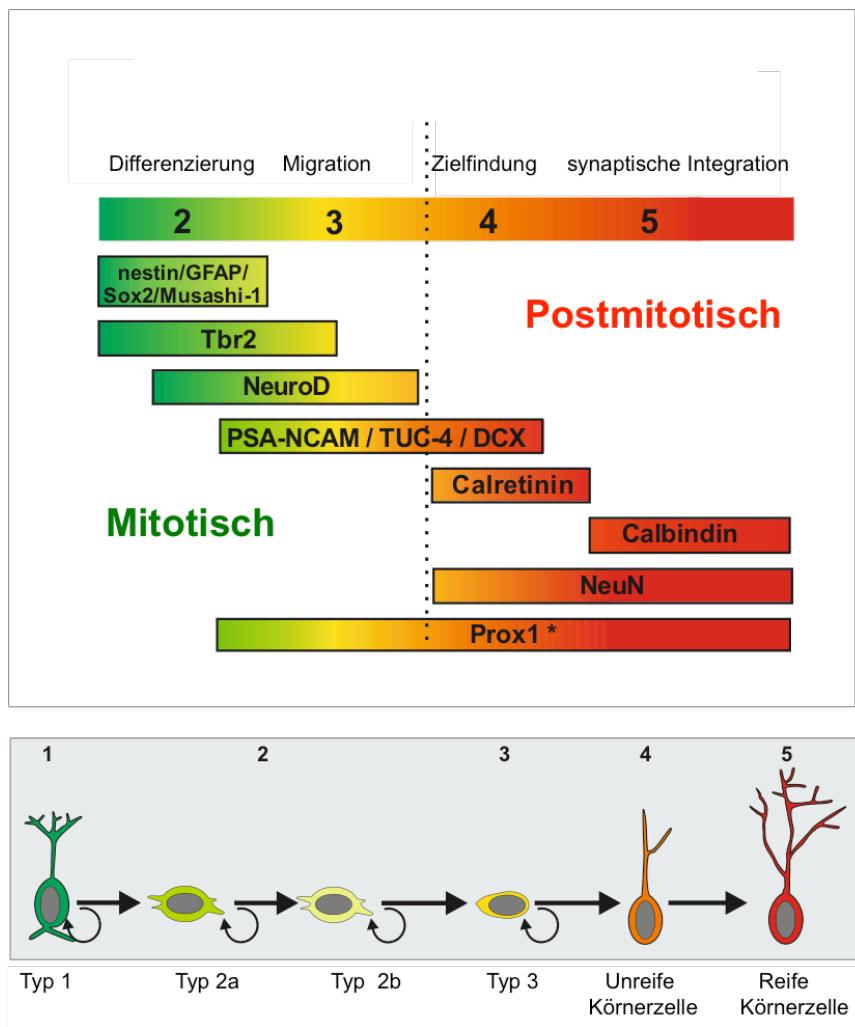


Abbildung 5: Übersicht über die Entwicklungsstadien der adulten Neurogenese und der in den verschiedenen Phasen exprimierten Marker.
(modifiziert nach (von Bohlen und Halbach, 2011))

2.2.2. Versuchsprotokolle

Für die Durchführung der Immunfluoreszenz-Färbungen, der Golgi-Imprägnation, der Dornen- und Zellzählung, der Messung des Volumens und der Schichtdicke des *Gyrus dentatus* und der statistischen Auswertung sei auf die angefügten Publikationen (Dokter et al., 2015, Poser et al., 2015) verwiesen.

3. Ergebnisse

An dieser Stelle werden die wesentlichen selbst erhobenen Ergebnisse aus den angefügten Publikationen zusammenfassend wiedergegeben.

3.1. Morphologie des Hippocampus

Um eine Übersicht über mögliche Veränderungen in der Schichtung des Hippocampus zu erhalten, wurden zunächst die Dicken des *Stratum granulosum* und des *Stratum moleculare* gemessen und das Volumen des *Gyrus dentatus* bestimmt.

3.1.1. Morphologie des Hippocampus im p75NTRE_{III} Knockout

In den adulten p75NTRE_{III}^{-/-}-Tieren zeigte sich eine signifikante Zunahme des Volumens des *Gyrus dentatus* (+ 12,8 %, je Gruppe N=3) und eine signifikante Zunahme der Dicke des *Stratum granulosum* (Kontrolle: 79,07 µm ± 2,96 µm, N=7 | p75NTRE_{III}^{-/-}: 86,89 µm ± 2,77 µm, N=7) im Vergleich zu altersentsprechenden Kontrollen des gleichen Mausstammes (Abbildung 6). Die Dicke des *Stratum moleculare* (Kontrolle: 223,04 µm ± 2,93 µm, N=7 | p75NTRE_{III}^{-/-}: 219,06 µm ± 6,66 µm, N=7) war unbeeinflusst vom p75NTRE_{III} Knockout (Dokter et al., 2015).

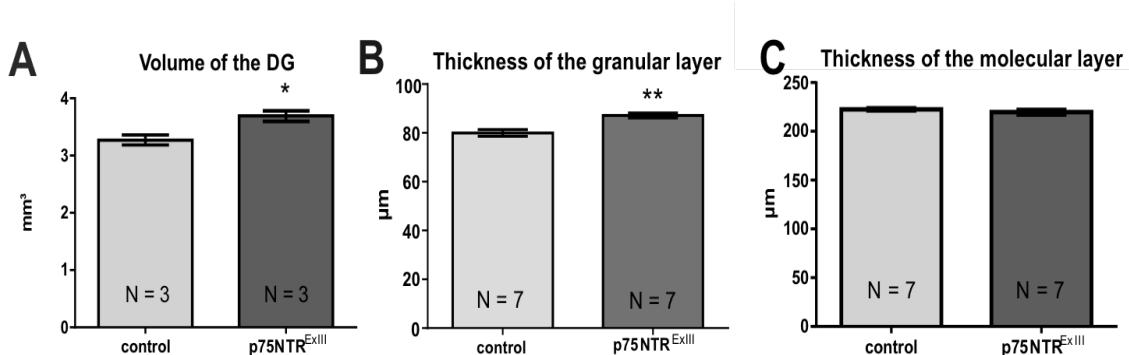


Abbildung 6: Volumen und Dicke des *Stratum granulosum* und des *Stratum moleculare* des *Gyrus dentatus*

Das Volumen des DG (engl. dentate gyrus = *Gyrus dentatus*) ist in den adulten p75NTRE_{III}^{-/-}-Mäusen im Vergleich zu altersentsprechenden Kontrolltieren signifikant erhöht (A). Ebenso ist in diesem Vergleich die Dicke des *Stratum granulosum* signifikant erhöht (B). Die Dicke des *Stratum moleculare* ist in den adulten p75NTRE_{III}^{-/-}-Tieren unverändert im Vergleich zu den altersentsprechenden Kontrolltieren. t-Test *p ≤ 0,05; **p ≤ 0,001 (nach (Dokter et al., 2015)).

3.2. Adulte Neurogenese im *Gyrus dentatus*

Aufgrund der erhöhten Dicke des *Stratum granulosum* und der verstärkten cholinergen Innervation der subgranulären Schicht des *Gyrus dentatus* (Dokter et al., 2015) wurde die Auswirkung des p75NTRExIII Knockouts auf die adulte Neurogenese genauer untersucht, denn eine erhöhte cholinerge Innervation des Hippocampus wird mit einer Steigerung der adulten Neurogenese in Verbindung gebracht (Kaneko et al., 2006).

3.2.1. Adulte Neurogenese im p75NTRExIII Knockout

Die Untersuchung der Proliferation mittels Zählung der phospho-Histon3-positiven Zellen (Kontrolle: $3093 \pm 283,9$ | p75NTRExIII^{-/-}: $2648 \pm 37,06$) im *Gyrus dentatus* zeigte weder in adulten noch in gealterten Mäusen einen signifikanten Unterschied im Vergleich zu altersentsprechenden Kontrolltieren des gleichen Mausstammes (je Gruppe N=4) (Abbildung 7, E). Die Zellproliferation scheint demnach unbeeinflusst vom p75NTRExIII Knockout zu sein (Dokter et al., 2015).

Da Proliferationsmarker wie phospho-Histon3, aber auch Ki67 oder BrdU keine Unterscheidung zwischen neuronaler und glialer Zelllinie erlauben, ist es nötig, einen zeitlich späteren Schritt der adulten Neurogenese zu untersuchen, um Aussagen über die Entstehung von neuen Neuronen treffen zu können (Dokter und von Bohlen und Halbach, 2012). Ein entsprechender Marker für die neuronale Linie ist das Protein Doublecortin (DCX), welches von mitotisch aktiven, jungen Neuronen und jungen postmitotischen Neuronen exprimiert wird (von Bohlen und Halbach, 2007). Der Vergleich adulter p75NTRExIII Knockouts mit altersentsprechenden Kontrolltieren zeigte keinen Unterschied hinsichtlich der Zahl DCX-positiver Zellen (Kontrolle: $8851 \pm 510,9$, N=7 | p75NTRExIII^{-/-}: $9207 \pm 792,8$, N=6). In gealterten p75NTRExIII Knockouts ($264,8 \pm 34,5$, N=4) zeigte sich eine leichte Reduktion der DCX-positiven Zellen im Vergleich zu altersentsprechenden Kontrollen ($411,5 \pm 38,5$, N=4); dieser Effekt war statistisch nicht signifikant (Abbildung 7, F). Wie erwartet, zeigte sich für beide Genotypen ein starker signifikanter altersabhängiger Effekt (Dokter et al., 2015).

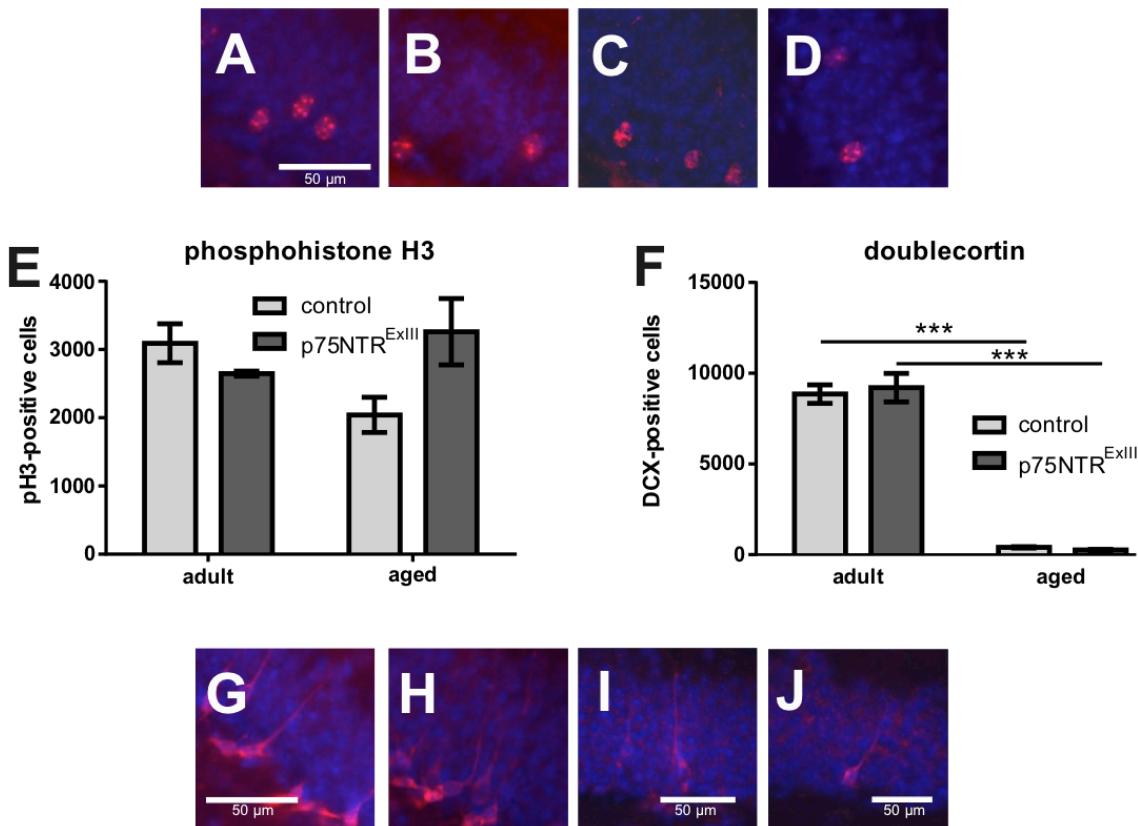


Abbildung 7: Anzahl der phospho-Histone H3- und DCX-positiven Zellen im Gyrus dentatus.
 Phospho-Histone H3-positive Zellen (rot) in einer adulten p75NTRExIII^{+/+} (A), einer adulten p75NTRExIII^{-/-} (B), einer gealterten p75NTRExIII^{+/+} (C) und einer gealterten p75NTRExIII^{-/-} Maus (D). Zellkerne sind mit DAPI (blau) dargestellt. [Maßstab A - D: 50 µm]. (E) Sowohl adulte als auch gealterte p75NTRExIII Knockouts zeigen keine Änderung in der Zahl phospho-Histone H3-positiver Zellen im Vergleich mit altersentsprechenden Kontrolltieren (*Two-way ANOVA*, gefolgt von *Tukey's multiple comparison Test*). (F) Die Zahl DCX-positiver Zellen zeigte beim Vergleich der p75NTRExIII Knockouts mit altersentsprechenden Kontrolltieren weder bei den adulten, noch bei den gealterten Tieren einen Unterschied. Jedoch zeigte sich ein starker altersabhängiger Effekt in beiden Genotypen (*Two-way ANOVA*, gefolgt von *Tukey's multiple comparison Test*). DCX-positive Zellen (rot) in einer adulten p75NTRExIII^{+/+} (G), einer adulten p75NTRExIII^{-/-} (H), einer gealterten p75NTRExIII^{+/+} (I) und einer gealterten p75NTRExIII^{-/-} Maus (J). Zellkerne sind mit DAPI (blau) dargestellt. [Maßstab G - J: 50 µm]. ***p ≤ 0,001 (nach (Dokter et al., 2015)).

3.3. Apoptose

Da Neurotrophine über den p75NTR auch Apoptose in hippocampalen Neuronen induzieren können (Troy et al., 2002), war in den p75NTR Knockouttieren eine reduzierte Apoptoserate im Hippocampus zu vermuten. In einer Studie aus dem Jahr 2008 zeigte sich entgegen dieser Vermutung eine Erhöhung der Apoptoserate (Catts et al., 2008). Aus diesem Grund analysierten wir die Zahl der apoptotischen Zellen im Hippocampus mittels Markierung von

Ergebnisse

aktivierter Caspase 3. Diese Versuche wurden bereits im Rahmen meiner Bachelorarbeit begonnen. Jetzt sollen sie in ergänzter und überarbeiteter Form vorgestellt werden.

3.3.1. Apoptose im p75NTRExIII Knockout

Im Vergleich von adulten p75NTRExIII Knockouts und altersentsprechenden Kontrolltieren (Abbildung 8, C) des gleichen Mausstammes (je Gruppe N=4) zeigte sich eine signifikante Verringerung der Apoptoserate um 17 % im *Gyrus dentatus* (Dokter et al., 2015).

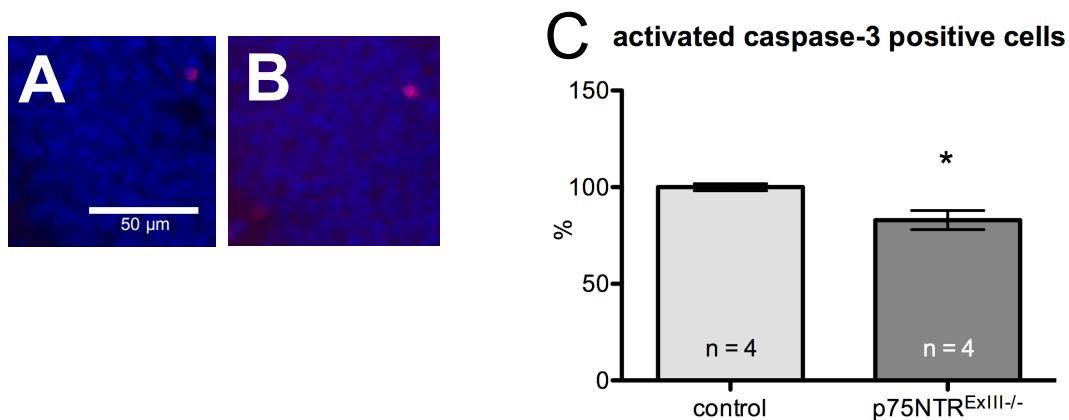


Abbildung 8: Anzahl aktiver Caspase3-positiver-Zellen im *Gyrus dentatus* des p75NTRExIII Knockouts.

Aktivierte Caspase3-positive-Zelle (rot) in einer adulten p75NTRExIII^{+/+} (A) und einer adulten p75NTRExIII^{-/-}-Maus (B). Zellkerne sind mit DAPI (blau) dargestellt. Maßstabsbalken A und B: 50 µm. Die Zahl aktiver Caspase3-positiver-Zellen ist reduziert in adulten p75NTRExIII Knockouts im Vergleich zu altersentsprechenden Kontrolltieren, [Maßstab A + B: 50 µm], t-Test * p ≤ 0,05 (nach (Dokter et al., 2015)).

3.3.2. Apoptose im p75NTRExIV Knockout

Im Vergleich der adulten p75NTRExIV^{-/-} Tiere mit altersentsprechenden Kontrolltieren des gleichen Mausstammes (je Gruppe N=6) zeigte sich eine signifikante Reduktion der Apoptoserate im *Gyrus dentatus* um 29 % (Abbildung 9, A). Dieser Unterschied war stärker ausgeprägt als im Vergleich der adulten p75NTRExIII^{-/-} Tiere mit altersentsprechenden Kontrolltieren (Abbildung 8, C) (Poser et al., 2015). Die Untersuchung der Zahl aktiver Caspase3-positiver-Zellen in gealterten p75NTRExIV^{-/-}-Tieren und altersentsprechenden Kontrolltieren (je Gruppe N=4) zeigte eine dezent

Ergebnisse

verringerte Anzahl aktiver Caspase3-positiver-Zellen im *Gyrus dentatus* (Abbildung 9, B). Aufgrund der starken Varianz, welche bei den gealterten Tieren in beiden Gruppen zu beobachten war, zeigte dieser Effekt keine statistische Signifikanz (Poser et al., 2015).

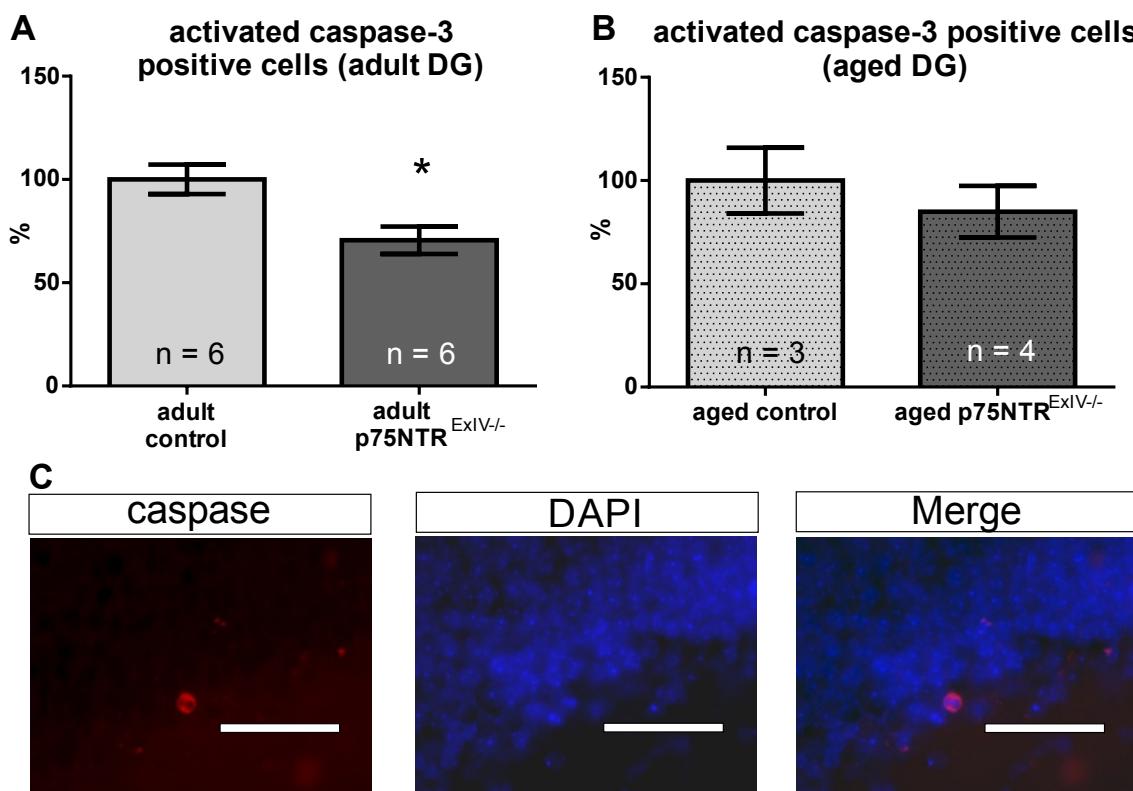


Abbildung 9: Anzahl aktiver Caspase3-positiver-Zellen im *Gyrus dentatus* des p75NTRExIV Knockouts

Die Zahl aktiver Caspase3-positiver-Zellen war signifikant erniedrigt in adulten p75NTRExIV Knockout-Tieren im Vergleich zu altersentsprechenden Kontrolltieren (A). Gealterte p75NTRExIV Knockout-Tiere zeigten einen dezenteren Effekt, welcher statistisch nicht signifikant war (B). Beispielhafte Darstellung (C) einer aktivierte Caspase 3-positiven Zelle (rot) im DG (engl. *dentate gyrus* = *Gyrus dentatus*). Zellkerne sind in DAPI (blau) dargestellt. In Merge sind die Farbkanäle blau und rot überlagert dargestellt; [Maßstabsbalken: 50 µm]; t-Test * p ≤ 0,05 (nach (Poser et al., 2015)).

3.4. Dendritische Dornen der Körnerzellen im *Gyrus dentatus*

In bereits publizierten Untersuchungen an Schnittkulturen des Hippocampus (sogenannte *organotypic slice cultures*) aus p75NTRExIII^{-/-} und p75NTRExIV^{-/-} Mäusen, konnte gezeigt werden, dass der p75NTR die Dichte der dendritischen Dornen in der CA1 Region des *Cornu ammonis* negativ beeinflusst (Zagrebelsky et al., 2005). Aufgrund dessen untersuchten wir nun den Einfluss

Ergebnisse

des p75 Knockouts auf die Dichte und Länge der dendritischen Dornen der Körnerzellen im *Gyrus dentatus*.

3.4.1. Dendritische Dornen im p75NTRExIII Knockout

Die Dichte der dendritischen Dornen der Körnerzellen im *Gyrus dentatus* war im Vergleich von p75NTRExIII^{-/-} Tieren und altersentsprechenden Kontrolltieren des gleichen Mausstammes (Kontrolle: $1,75 \text{ /}\mu\text{m} \pm 0,03 \text{ /}\mu\text{m}$, N=6 | p75NTRExIII^{-/-}: $1,91 \text{ /}\mu\text{m} \pm 0,04 \text{ /}\mu\text{m}$, N=5) signifikant erhöht (Abbildung 10, A). Die Untersuchung der mittleren Länge der dendritischen Dornen (Kontrolle: $1,5 \text{ }\mu\text{m} \pm 0,18 \text{ }\mu\text{m}$, N=6 | p75NTRExIII^{-/-}: $1,38 \text{ }\mu\text{m} \pm 0,26 \text{ }\mu\text{m}$, N=5) erbrachte keinen Unterschied im Vergleich von p75NTRExIII^{-/-} Tieren und altersentsprechenden Kontrolltieren des gleichen Mausstammes (Abbildung 10, B)(Dokter et al., 2015).

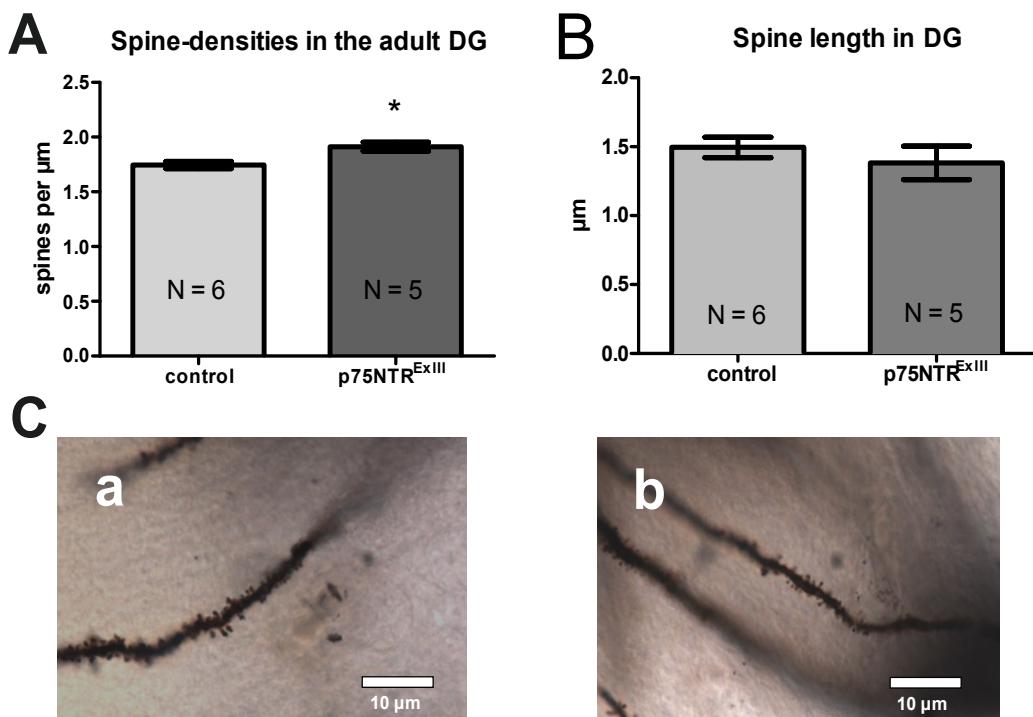


Abbildung 10: Dichte und Länge der dendritischen Dornen im p75NTRExIII Knockout

Im Vergleich der p75NTRExIII^{-/-}-Tiere mit altersentsprechenden Kontrolltieren war die Dichte der dendritischen Dornen signifikant erhöht (A), während die mittlere Länge der dendritischen Dornen keinen Unterschied zeigte (B). Beispielhafte Darstellung eines Dendriten im DG (engl. *dentate gyrus* = *Gyrus dentatus*) einer p75NTRExIII^{+/+} (C, a) und einer p75NTRExIII^{-/-} Maus (C, b) in Golgi-imprägnierten Schnitten mit 120 μm Schnittdicke. Da jeweils nur ein schmales Segment scharf sichtbar ist, wurden die Dendriten im dreidimensionalen Raum rekonstruiert. Objektiv: 100x Öl (NA: 1,4); Maßstabsbalken: 10 μm ; t-Test * $p \leq 0,05$. (nach (Dokter et al., 2015)).

3.4.2. Dendritische Dornen im p75NTRExIV Knockout

Im Gegensatz zu Tieren des p75NTRExIII Knockouts zeigen die Tiere des p75NTRExIV Knockouts keine Änderung bezüglich der Dichte der dendritischen Dornen der Körnerzellen (Kontrolle: $1,73 \text{ /}\mu\text{m} \pm 0,03 \text{ /}\mu\text{m}$, N=4 | p75NTRExIII^{-/-}: $1,79 \text{ /}\mu\text{m} \pm 0,13 \text{ /}\mu\text{m}$, N=4) des *Gyrus dentatus* (Abbildung 11, A). Ebenso zeigte auch die mittlere Länge der dendritischen Dornen der Körnerzellen im *Gyrus dentatus* (Kontrolle: $1,29 \mu\text{m} \pm 0,07 \mu\text{m}$, N=4 | p75NTRExIV^{-/-}: $1,22 \mu\text{m} \pm 0,08 \mu\text{m}$, N=4) keinen signifikanten Unterschied (Abbildung 11, B) (Poser et al., 2015).

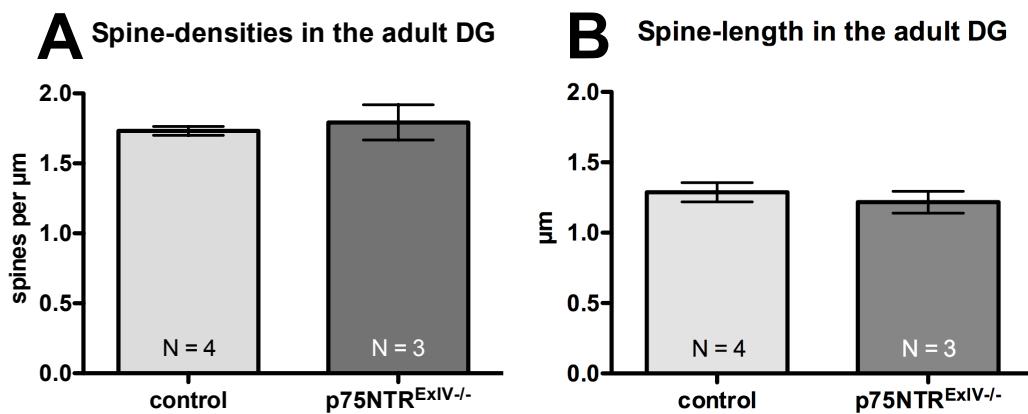


Abbildung 11: Dichte und Länge der dendritischen Dornen im p75NTRExIV Knockout

Die Dichte der dendritischen Dornen der Körnerzellen im DG (engl. *dentate gyrus* = *Gyrus dentatus*) zeigte beim Vergleich zwischen p75NTRExIV^{-/-} Mäusen und altersentsprechenden Kontrolltieren keinen Unterschied (A). Ebenso war die mittlere Länge der dendritischen Dornen der Körnerzellen im *Gyrus dentatus* nicht vom p75NTRExIV Knockout beeinflusst (B) (nach (Poser et al., 2015)).

4. Diskussion

In zahlreichen bisher publizierten Studien konnte gezeigt werden, dass der Knockout des p75NTR im Hippocampus einen Einfluss auf die Morphologie, die cholinerge Innervation, die adulte Neurogenese, die Spinogenese und bestimmtes hippocampus-assoziertes Verhalten hat (Naumann et al., 2002, Zagrebelsky et al., 2005, Catts et al., 2008, Barrett et al., 2010, Bernabeu und Longo, 2010, Colditz et al., 2010, Greferath et al., 2012). Die meisten dieser Studien wurden jedoch am p75NTRExIII Knockout durchgeführt und führten zu teilweise gegensätzlichen Ergebnissen. Die Tiere dieses Knockouts exprimieren weiterhin die verkürzte Splicevariante des p75NTR. Daher soll in der vorliegenden Arbeit der Einfluss des p75NTRExIII Knockouts auf oben genannte Faktoren reevaluiert und der Einfluss des vollständigen Fehlens beider Splicevarianten des p75NTR auf diese Faktoren im p75NTRExIV Knockout untersucht werden.

In beiden p75NTR Knockoutlinien konnten wir zeigen, dass die Morphologie des Hippocampus im Vergleich zu Wildtyptieren verändert ist. Insbesondere war sowohl in den p75NTRExIII als auch in den p75NTRExIV Knockouts das *Stratum granulosum* des *Gyrus dentatus* verbreitert (Dokter et al., 2015). In den p75NTRExIV Tieren war darüber hinaus auch eine Verbreiterung des *Stratum moleculare* des *Gyrus dentatus* zu beobachten (Poser et al., 2015). Da der Hippocampus eine Hirnstruktur mit lebenslanger regenerativer Kapazität ist (Bruel-Jungerman et al., 2011), untersuchten wir darauf aufbauend die möglichen morphologischen Korrelate dieser Veränderungen in adulten und gealterten p75NTR Knockouttieren.

Der Hippocampus erhält seine cholinerge Innervation hauptsächlich aus dem cholinergen System des basalen Vorderhirns und hier besonders aus den medialen Septumkernen (Niewiadomska et al., 2011). Es wird vermutet, dass unter anderem das cholinerge System die adulte Neurogenese positiv beeinflusst (Bruel-Jungerman et al., 2011). Für beide p75NTR Knockouts ist bekannt, dass sie im Vergleich mit Wildtyptieren eine erhöhte Anzahl cholinriger Zellen im Septum haben. Im Fall der p75NTRExIV Knockouts war

Diskussion

diese Steigerung stärker ausgeprägt als bei den p75NTRE_{III} Knockouts (Naumann et al., 2002). Wir konnten zeigen, dass die cholinerge Innervation des Hippocampus in den p75NTR Knockouts gegenüber den Wildtypieren erhöht ist (Dokter et al., 2015, Poser et al., 2015). Interessanterweise blieb in den p75NTRE_{IV} Knockouts die Erhöhung der cholinergen Innervation auch im Alter bestehen (Poser et al., 2015). Es ist zu vermuten, dass die Erhöhung der cholinergen Innervation Folge der Erhöhung der Zahl cholinriger Zellen im Septum ist.

Es ist bekannt, dass Acetylcholin mit Lernen und Gedächtnis (Deiana et al., 2011) und Defekte des cholinergen Systems mit Altern und Alzheimer assoziiert sind (Muir, 1997). Weiterhin ist bekannt, dass Gehirne, welche von Alzheimer betroffen sind, eine schwere Reduktion des cholinergen Systems aufweisen (Terry und Davies, 1980). Außerdem wird dem p75NTR eine Rolle in der Pathogenese der Alzheimer Krankheit zugesprochen. Demnach ist der p75NTR in Alzheimerpatienten im Hippocampus verstärkt exprimiert, an der Formation der sogenannten *Neurofibrillären Tangles* beteiligt und stimuliert die Genese der Amyloidplaques (Hu et al., 2002, Chakravarthy et al., 2012). Unsere aktuellen Daten lassen vermuten, dass das vollständige Fehlen beider Splicevarianten des p75NTR die gealterten Tiere vor der altersassoziierten Reduktion des cholinergen Systems bewahrt haben. Es wäre nun von großer Relevanz zu untersuchen, ob ein p75NTR Knockout die Bildung der sogenannten *Neurofibrillären Tangles* in der Pathogenese der Alzheimer Krankheit verhindern kann.

Während des gesamten Lebens entwickeln sich im Hippocampus Neuronen aus neuronalen Vorläuferzellen. Diese Entwicklung ist von verschiedenen intrinsischen und extrinsischen Faktoren beeinflusst (Kempermann, 2011). Eine Reduktion der adulten Neurogenese scheint weiterhin mit Störungen des Gedächtnisses und des räumlichen Lernens assoziiert zu sein (Dokter und von Bohlen und Halbach, 2012)

In der Vergangenheit konnte mehrfach gezeigt werden, dass das cholinerge System die adulte Neurogenese positiv beeinflusst (Mohapel et al., 2005,

Diskussion

Kaneko et al., 2006). Außerdem ist bekannt, dass der Wachstumsfaktor *nerve growth factor* (NGF) das Überleben von neu entstandenen Neuronen im Hippocampus von Ratten fördert, wenn dieser ihnen intraventrikulär infundiert wird (Frielingsdorf et al., 2007). NGF wiederum ist der Hauptwachstumsfaktor für die cholinergen Zellen des Septums, welche wiederum den Hippocampus innervieren (Hefti und Will, 1987). Es ist daher zu vermuten, dass unter anderem die Wirkung des NGF über den p75NTR eine Rolle in der Regulation der adulten Neurogenese spielt.

Eine weitere Hirnregion, in der postnatal Neurogenese stattfindet, ist die Subventrikularzone. Die hier entstehenden Neuronen wandern über den sogenannten *rostral migratory stream* in den *Bulbus olfactorius* und ersetzen dort Interneuronen. Die hoch proliferativen Zellen in dieser Hirnregion exprimieren den p75NTR und es wird vermutet, dass der Signalweg dieses Rezeptors die Neurogeneserate in der Subventrikularzone beeinflusst (Gascon et al., 2007, Young et al., 2007). Wir vermuteten, dass der p75NTR auch die adulte Neurogenese im *Gyrus dentatus* beeinflusst. In anderen Studien konnte diesbezüglich gezeigt werden, dass Neurotrophine über den p75NTR in hippocampalen Neuronen Zelltod induzieren (Troy et al., 2002) und dass die Vorstufe des Wachstumsfaktors NGF, proNGF, über den p75NTR die adulte Neurogenese im Hippocampus reduziert (Guo et al., 2013). Unsere Hypothese war hier, dass das Fehlen des p75NTR die adulte Neurogenese im Hippocampus erhöht. Dem gegenüber stehen Untersuchungen, die eine Reduktion der adulten Neurogenese in p75NTR Knockouttieren beschrieben haben (Catts et al., 2008, Bernabeu und Longo, 2010, Colditz et al., 2010). Diese Untersuchungen fanden unter Verwendung des p75NTRExIII Knockouts statt, in dem weiterhin eine verkürzte Variante des p75NTR exprimiert wird.

Unsere Untersuchungen bezüglich der Neurogenese in adulten und gealterten p75NTRExIII Knockouttieren zeigten keine signifikante Reduktion der adulten Neurogenese. Auch die Arbeit der Gruppe um Martinowich kommt zu diesem Ergebnis (Martinowich et al., 2012). Da die Tiere des p75NTRExIII Knockouts weiterhin die verkürzte Variante des p75NTR ohne Neurotrophinbindestelle exprimieren, untersuchten wir zusätzlich p75NTRExIV^{-/-} Tiere, um einen

Diskussion

möglichen Effekt des verkürzten Rezeptors zu detektieren. In diesen Untersuchungen zeigte sich kein Effekt des vollständigen p75NTR Knockouts auf die Proliferation, wohl aber auf die Differenzierung der neu entstandenen Neuronen im *Gyrus dentatus*. Hier konnten wir zeigen, dass die Zahl der Doublecortin-postiven-Zellen in den adulten p75NTRExIV Knockouts im Vergleich mit entsprechenden Kontrolltieren signifikant erhöht war. Eine solche Erhöhung zeigte sich in den gealterten Tieren des gleichen Stammes nicht. Darüber hinaus waren die Dendritenbäume dieser Doublecortin-postiven-Zellen komplexer als die entsprechender Wildtypmäuse. Dies deutet auf eine veränderte neuronale Differenzierung in den p75NTRExIV Knockouts hin. Interessanterweise konnten wir nur in den Tieren eine derartige Änderung beschreiben, in denen sowohl die verkürzte als auch die normale Variante des p75NTR fehlte (p75NTRExIV). Es ist demnach zu vermuten, dass hier mögliche neurotrophinunabhängige Effekte über den verkürzten p75NTR eine Rolle spielen. Auch scheint es in unserem Fall keinen eindeutigen Zusammenhang zwischen der verstärkten cholinergen Innervation und der Steigerung der adulten Neurogenese zu geben, denn nur für die adulten p75NTRExIV Knockouts zeigte sich eine entsprechende Korrelation.

Wie bereits erwähnt, sind proapoptotische Signale des p75NTR bekannt. Über die Bindung von Neurotrophinen und die nachfolgende Aktivierung von JNK, p53, Bax und schließlich von Caspasen werden über den p75NTR entsprechende Signale vermittelt (Underwood und Coulson, 2008). Eine Reduktion der Apoptoserate in den p75NTR Knockouts war demnach zu erwarten. Diesem Befund widersprechend, existiert eine Studie aus dem Jahr 2008, in der genau das Gegenteil, nämlich ein Anstieg der Apoptoserate im p75NTRExIII Knockout beschrieben wird (Catts et al., 2008). Hier ist zu beachten, dass es sich bei der Untersuchung der Apoptose mittels Immunfluoreszenz um eine Momentaufnahme zu einem bestimmten Zeitpunkt handelt. Unsere Daten belegen eine Reduktion der Apoptoserate, sowohl in den p75NTRExIII als auch in den p75NTRExIV Knockouttieren. Jedoch zeigte sich, dass diese Reduktion in den p75NTRExIV Knockouts etwa doppelt so stark ausgeprägt war wie bei den p75NTRExIII Knockouts ($p75\text{NTRExIII}^{-/-}$ – 17 % | $p75\text{NTRExIV}^{-/-}$ – 29 %). Auch hier kann wieder vermutet werden, dass von

Diskussion

der verkürzten Variante des p75NTR eine proapoptotische Funktion, die neurotrophinunabhängig ist, ausgeht. Denn erst in den p75NTRExIV Knockouts fehlt sowohl die verkürzte als auch die normale Version des p75NTR.

Ausgehend von einem möglichen Einfluss des p75NTR auf die Differenzierung von neu entstandenen Neuronen und im Hinblick auf die Ergebnisse der Arbeitsgruppe um Marta Zagrebelsky, die eine vergrößerte Dichte der dendritischen Dornen in den Pyramidenzellen des *Cornu ammonis* beschrieben hat, untersuchten wir mithilfe der Golgi-Imprägnation die Dichte der dendritischen Dornen der Körnerzellen des *Gyrus dentatus*. Hier zeigte sich ein signifikanter Anstieg der Dichte der dendritischen Dornen in den adulten p75NTRExIII Knockouts. Im p75NTRExIV Knockout konnten wir lediglich eine altersabhängige Reduktion der Dichte der dendritischen Dornen, sowohl bei den Kontroll- als auch bei den Knockouttieren, zeigen.

Neben den morphologischen Veränderungen durch den Verlust des p75NTR wurden für beide Knockouts auch Änderungen bezüglich elektrophysiologischer Parameter beschrieben. Die sogenannte *long-term depression* (LTD) betreffend wurde gezeigt, dass diese in den p75 Knockouts beeinträchtigt ist (Rosch et al., 2005). Für die sogenannte *long-term potentiation* (LTP) existieren gegensätzliche Befunde. So ist einerseits eine Steigerung (Barrett et al., 2010) andererseits kein Effekt auf die LTP (Rosch et al., 2005) beschrieben. Sowohl LTP als auch LTD sind Formen langdauernder synaptischer Plastizität und werden mit Lern- und Gedächtnisvorgängen in Zusammenhang gebracht (Bach et al., 1995). Für die LTD ist beschrieben, dass es einen funktionellen Zusammenhang mit der Konsolidierung des räumlichen Gedächtnisses gibt (Ge et al., 2010) und das die LTD einen Einfluss auf das Verhalten von Mäusen im sogenannten *Morris water maze* hat (Dong et al., 2013). Diese Ergebnisse beachtend, sollte für beide p75 Knockout Linien untersucht werden, ob ein Einfluss auf das Hippocampus-assoziierte Verhalten festzustellen ist. Aufgrund der schweren Ataxie der hinteren Extremitäten bei den Tieren des p75NTRExIV Knockouts (von Schack et al., 2001) konnten diese Tiere nicht mit den Verhaltenstests untersucht werden, da hierfür meist eine normale Bewegungsfähigkeit der Tiere erforderlich ist. Die Untersuchung der p75NTRExIII Knockouts im *Morris water*

Diskussion

maze zeigte in der Trainingsphase keine Unterschiede zwischen Knockout und Wildtyp. Jedoch sahen wir in der Kontrolluntersuchung ein signifikant schlechteres Abschneiden der p75NTRE_{III} Knockouttiere im Vergleich mit entsprechenden Kontrolltieren. Dies werteten wir als Hinweis auf eine Störung der Speicherung von neuen Inhalten in das räumliche Gedächtnis (Dokter et al., 2015). Ähnliche Ergebnisse konnten unlängst bei der Verwendung von Mäusen mit einem konditionellen Knockout des Wachstumsfaktors BDNF erzielt werden. Auch hier zeigten sich lediglich in der Kontrolluntersuchung des *Morris water maze* Auffälligkeiten (Vigers et al., 2012). Diese Parallele deutet auf eine Beteiligung des p75NTR an BDNF vermittelten Lern- und Gedächtnisvorgängen hin.

Wie bereits mehrfach erwähnt, exprimieren die Tiere des p75NTRE_{III} Knockouts weiterhin eine verkürzte Splicevariante des p75NTR, von der möglicherweise neurotrophinunabhängige Signale ausgehen. Auch muss beachtet werden, dass der p75NTR vielfältige Rezeptoren als Kooperationspartner nutzt (Trk, SorCS2, Nogo Rezeptor) (Wang et al., 2002, Chao, 2003, Nykjaer et al., 2004) und der Mechanismus der Aktivierung und Signaltransduktion bisher weitgehend unklar ist. Erst kürzlich konnte gezeigt werden, dass der Status des p75NTR als Trimer oder Monomer und seine Expressionsrate wesentliche funktionelle, zum Teil unterschiedliche, Auswirkungen haben (Anastasia et al., 2015).

Durch die vorliegende Arbeit wurden einige wesentliche Parameter der Morphologie des Hippocampus von adulten und gealterten p75NTR defizienten Mäusen bestätigt, zum Teil korrigiert aber auch neu untersucht. Insbesondere konnten wir zeigen, dass es hier bedeutsame Unterschiede zwischen beiden p75NTR Knockoutlinien gibt. Diese betreffen die Dichte der dendritischen Dornen einerseits und die adulte Neurogenese andererseits. Weiterhin sind auch die Effekte des Alters in beiden Mauslinien zum Teil unterschiedlich (Dichte der dendritischen Dornen, cholinerge Innervation). Der p75NTR scheint also eine Rolle in der Regulation des cholinergen Systems, der Spinogenese und der adulten Neurogenese im *Gyrus dentatus* zu spielen.

5. Zusammenfassung

Der p75 Neurotrophinrezeptor (p75NTR) bindet alle Neurotrophine (NGF, BDNF, NT3, NT4). Er wird in der Entwicklung sehr stark, im adulten Gehirn nur noch punktuell exprimiert. Nichtsdestotrotz existieren zahlreiche Studien, die eine Rolle dieses Rezeptors für die Morphologie und Funktion des Hippocampus implizieren. Überraschenderweise sind diese Daten zum Teil widersprüchlich. Zudem wurde bisher fast ausschließlich die p75NTRExIII Knockout Maus verwendet, die jedoch weiterhin die verkürzte Splicevariante des p75NTR ohne Neurotrophinbindestelle exprimiert. Die vorliegende Arbeit soll nun einerseits dazu beitragen, die bestehenden Daten bezüglich des p75NTRExIII zu reevaluieren und anderseits mithilfe des p75NTRExIV Knockouts die Auswirkungen des vollständigen Fehlens des p75NTR auf die Morphologie des Hippocampus zu untersuchen. So konnten wir zeigen, dass sowohl das Fehlen der verkürzten, als auch beider Splicevarianten, die Morphologie des Hippocampus und dessen cholinerge Innervation verändert. Ferner stellte sich heraus, dass nur das Fehlen beider Splicevarianten einen Einfluss auf die adulte Neurogenese hat, jedoch in beiden Knockoutlinien erniedrigte Apoptoseraten im Hippocampus ermittelt werden konnten. Hinsichtlich der dendritischen Dornen ist nur in den p75NTRExIII Knockouts ein Anstieg ihrer Dichte feststellbar. Diese morphologischen Veränderungen waren, zumindest im Fall der p75NTRExIII Knockouts, von verändertem Verhalten begleitet. Aufgrund der schweren Ataxie der hinteren Extremitäten bei den p75NTRExIV Knockouts, konnten lediglich die p75NTRExIII Knockouts und entsprechende Kontrolltiere bezüglich des Verhaltens untersucht werden. Hier zeigte sich eine deutliche Einschränkung in der Speicherung des räumlichen Gedächtnisses. Unsere Daten belegen, dass sowohl das Fehlen der verkürzten als auch beider Varianten des p75NTR einen Einfluss auf die Morphologie und die Funktion des Hippocampus hat.

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7. Anhang

7.1. Abbildungsverzeichnis

Abbildung 1: Schichten des Hippocampus in der Nissl-Färbung.....	1
Abbildung 2: Schematische Übersicht der Verbindungen innerhalb des medialen Temporallappens.....	2
Abbildung 3: Der trisynaptische Kreis; Synaptische Verbindungen in der Hippocampusformation.....	3
Abbildung 4: Der p75 Rezeptor in den verfügbaren Mauslinien.....	13
Abbildung 5: Übersicht über die Entwicklungsstadien der adulten Neurogenese und der in den verschiedenen Phasen exprimierten Marker.....	15
Abbildung 6: Volumen und Dicke des <i>Stratum granulosum</i> und des <i>Stratum moleculare</i> des <i>Gyrus dentatus</i>	17
Abbildung 7: Anzahl der phospho-Histon3- und DCX-positiven Zellen im <i>Gyrus dentatus</i>	19
Abbildung 8: Anzahl aktiver Caspase3-positiver-Zellen im <i>Gyrus dentatus</i> des p75NTRExIII Knockouts	20
Abbildung 9: Anzahl aktiver Caspase3-positiver-Zellen im Gyrus dentatus des p75NTRExIV Knockouts	21
Abbildung 10: Dichte und Länge der dendritischen Dornen im p75NTRExIII Knockout	22
Abbildung 11: Dichte und Länge der dendritischen Dornen im p75NTRExIV Knockout	23

7.2. Abkürzungsverzeichnis

°C	Grad Celsius
Akt	Gen der Proteinkinase B
ANOVA	<i>analysis of variance</i>
Apaf1	Apoptotic protease activating factor 1
Asp	Aspartat
Bad	<i>Bcl-2-Antagonist of Cell Death</i>
Bak	<i>Bcl-2 -antagonist/killer-1</i>
Bax	<i>Bcl-2-associated X protein</i>
Bcl2	B-cell lymphoma 2
BDNF	<i>brain-derivate neurotrophic factor</i>
BH3	<i>Bcl-2 homology domain 3</i>
Bid	<i>BH3 interacting-domain death agonist</i>
Bim	<i>Bcl-2 interacting mediator of cell death</i>
BrdU	Bromodesoxyurinin
C	Cystein
CA 1, 2, 3	Unterregionen des Cornu ammonis
CRMP	<i>Collapsin response mediator protein</i>
Cy3	Indocarbocyanin
DAPI	4',6-Diamidin-2-phenylindol
DCX	Doublecortin
DG	<i>Dentate gyrus für Gyrus dentatus</i>
DISC	<i>death-inducing-singaling-complex</i>
GFAP	<i>glial fibrillary acidic protein</i>
JNK	c-Jun N-terminale Kinasen
LTD	<i>long-term depression</i>
LTP	<i>long-term potentiation</i>
NeuN	<i>Neuronal Nuclei</i>
NeuroD	<i>Neurogenic differentiation</i>
NGF	<i>nerve growth factor</i>
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4

Pax6	<i>paired box protein 6</i>
Prox1	<i>prospero homeobox 1</i>
PSA-NCAM	<i>polysialylated embrionic form of the neural cell adhesion molecule</i>
PSD	Postsynaptische Densität
p75NTR	p75 Neurotrophinrezeptor
pH3	phosphoHiston3
SorCS2	<i>sortilin-related VPS10 domain containing receptor 2</i>
Sox2	<i>sex determining region Y (SRY)- box 2</i>
Tbr2	<i>T-box brain protein 2</i>
TNFR I	Tumornekrosefaktor Rezeptor I
TOAD	<i>turned on after division</i>
Trk	Tropomyosin-Rezeptor Kinase
TUC-4	Mitglied der TOAD/Ulip/CRMP-Familie
Ulip	<i>Unc-33-like phosphoprotein</i>

7.3. Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät, keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, 13.11.2015

Martin Dokter

7.4. Lebenslauf

7.5. Liste der Veröffentlichungen

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Dokter, M., Busch, R., Poser, R., Vogt, M. A., von Bohlen Und Halbach, V., Gass, P., Unsicker, K. and von Bohlen Und Halbach, O. (2015). Implications of p75NTR for dentate gyrus morphology and hippocampus-related behavior revisited. *Brain Struct Funct* 220(3): 1449-1462, doi: 10.1007/s00429-014-0737-5.

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7.5.2. Reviews

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7.5.3. Kongressbeiträge

Diana Brackrock, Martin Dokter, Klaus-Peter Philipp, Britta Bockholdt. *Was deckt die zweite Leichenschau im Krematorium auf?* 94. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin, 09/2015 Leipzig (Poster)

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Martin Dokter, Robert Poser, Ruben Busch, Miriam Vogt, Marian Baldus, Peter Gass, Oliver von Bohlen und Halbach. *Specific effects of the deletion of p75 receptors on the hippocampal formation.* Tagung der Neurowissenschaftlichen Gesellschaft, 03/13, Göttingen (Poster)

Martin Dokter, Oliver von Bohlen und Halbach. Knockout of the p75 neurotrophin receptor alters the differentiation of granule cells within the dentate gyrus. The 8th FENS Forum of Neuroscience, 06/2012, Barcelona, Spanien (Poster)

Martin Dokter, Oliver von Bohlen und Halbach. *Loss of the neurotrophin receptor p75NTR alters the morphology of the dentate gyrus.* 28. Arbeitstagung der Anatomischen Gesellschaft, 09/2011, Würzburg (Poster)

Martin Dokter, Oliver von Bohlen und Halbach. *P75NTR acts as a negative differentiation regulator in the adult dentate gyrus.* 27. Arbeitstagung der Anatomischen Gesellschaft, 09/2010, Würzburg (Poster)

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Implications of p75NTR for dentate gyrus morphology and hippocampus-related behavior revisited

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Abstract The pan-neurotrophin receptor p75NTR is expressed in the adult brain in a discrete pattern. Although numerous studies have addressed its implications for hippocampal functions, the generated sets of data are surprisingly conflicting. We have therefore set out to re-investigate the impact of a deletion of the full-length p75NTR receptor on several parameters of the dentate gyrus (DG), including neurogenesis and hippocampus-related behavior by using p75NTR^{ExIII} knockout mice. Moreover, we investigated further parameters of the DG (cholinergic innervation, dendritic spines). In addition, we analyzed on the morphological level the impact of aging by comparing adult and aged p75NTR^{ExIII} mice and their age-matched littermates. Adult (4–6 months old), but not aged (20 months old), p75NTR^{ExIII} knockout mice display an enhanced volume of the DG. However, adult neurogenesis within the adult DG was unaffected in both adult and aged p75NTR^{ExIII} knockout mice. We could further demonstrate that the change in the volume of the DG was accompanied by an increased cholinergic innervation and increased spine

densities of granule cells in adult, but not aged p75NTR deficient mice. These morphological changes in the adult p75NTR deficient mice were accompanied by specific alterations in their behavior, including altered behavior in the Morris water maze test, indicating impairments in spatial memory retention.

Keywords Neurotrophin · p75NTR · Adult neurogenesis · Dendritic spine · Morris water maze · Aging

Introduction

The neurotrophins [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4 (NT4)] bind to receptors that belong to the class of the Trk family of tyrosine protein kinase receptors (Chao 2003). NGF specifically recognizes trkB, whereas BDNF and NT-4 specifically activate trkB receptors. NT-3 primarily activates trkB receptors. In addition, all neurotrophins can signal through a low-affinity receptor, known as the p75NTR receptor. Two isoforms of the p75NTR receptor exist: a short (s-p75NTR) and a full-length isoform. The full-length isoform is capable of binding neurotrophins, whereas the short isoform lacks the neurotrophin binding site. Although the functions of s-p75NTR are largely unknown, some studies suggest that it is a functional receptor *in vivo* (Fujii and Kunugi 2009). In addition to mature neurotrophins, proneurotrophins (the uncleaved neurotrophin precursors) can be secreted and bind to a p75NTR–sortilin complex to induce, e.g., neuronal apoptosis (Lee et al. 2001; Teng et al. 2005).

The p75 receptor is expressed within the hippocampus (Barrett et al. 2005), including the dentate gyrus (DG). Several studies have assessed its putative impact on

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Table 1 Controversial data obtained in p75NTR^{ExIII} mice

	Number of cholinergic neurons (basal forebrain; septum)	Size of cholinergic neurons (basal forebrain; septum)	Adult hippocampal neurogenesis (BrdU measurements)	Spatial memory performance	Open-field locomotor activity
Increase	Greferath et al. (2012), Yeo et al. (1997), Van der Zee et al. (1996), Naumann et al. (2002)	Yeo et al. (1997), Greferath et al. (2012)			Greferath et al. (2000), Barrett et al. (2010)
Decrease	Peterson et al. (1999)		Catts et al. (2008), Bernabeu and Longo (2010), Colditz et al. (2010)	Wright et al. (2004)	Barrett et al. (2010)
No change	Greferath et al. (2000)	Ward et al. (2000)	Martinowich et al. (2012)	Catts et al. (2008)	Martinowich et al. (2012)

hippocampal morphology, cholinergic innervation, neurogenesis, and hippocampus-related behavior, with strikingly conflicting results (cf Table 1). For example, in hippocampal field CA1, p75NTR has been reported to act as a negative modulator of dendritic spine density, at least in vitro (Zagrebelsky et al. 2005). In contrast, analysis of dendritic spine density in slice cultures treated with the p75NTR function-blocking antibody REX has failed to reveal any effect on spine densities (Chapleau and Pozzo-Miller 2012).

With respect to adult neurogenesis, which occurs in the DG constitutively throughout postnatal life and is influenced by environment, behavior, and aging, it has been demonstrated that p75NTR is expressed by the majority of progenitor cells located in the subgranular zone (SGZ) of the DG and by the majority of cells belonging to the neuronal lineage (Bernabeu and Longo 2010). Several studies (cf. Table 1) have shown that elimination of p75NTR^{ExIII} results in a decline in BrdU-positive cells within the DG following long-term treatment with BrdU (Bernabeu and Longo 2010; Catts et al. 2008; Colditz et al. 2010). As an underlying mechanism, it has been hypothesized that loss of p75NTR promotes neuroblast cell death rather than survival (Catts et al. 2008). Alternatively, it has been suggested that loss of p75NTR reduces proliferation and early maturation of neuroblasts in the adult DG (Bernabeu and Longo 2010), collectively indicating that both the precise phenotype of adult neurogenesis in the p75-deprived DG and its underlying mechanisms still need to be clarified.

Apart from the hippocampus, cholinergic basal forebrain projection neurons are among the few populations that express p75NTR in the adult CNS. Cholinergic neurons located in the medial septal nucleus project to the hippocampal formation (Mufson et al. 2003). However, reports concerning an impact of p75NTR^{ExIII} deletion on CNS cholinergic neurons are also contradictory (Table 1). Likewise, data on the behavior of p75NTR^{ExIII} deficient mice are not consistent (Table 1). The conflicting reports on hippocampal structure and behavior of p75NTR^{ExIII}

deficient mice may be attributed to the application of diverse methods used for analysis as well as to the genetic background of the p75NTR^{ExIII} deficient mice and their controls. In several studies, the p75NTR^{ExIII} mice were backcrossed to Balb/c (Bernabeu and Longo 2010; Yeo et al. 1997), 129/SV (Barrett et al. 2010; Greferath et al. 2012) or interbred with C57Bl6/J (Catts et al. 2008; Martinowich et al. 2012). These different strategies may account for the discrepant data; for example, it has been shown that the distribution of cholinergic septal neurons in p75NTRE^{xIII} mice is influenced by the genetic background (Naumann et al. 2002). Interestingly, pure C57Bl6 animals have about 33 % fewer cholinergic neurons than Sv129 animals. This background-dependent effect is larger than the largest difference observed with the p75NTR mutant animals with different genetic background (Naumann et al. 2002).

During aging, there is a dramatic reduction in the rate of adult neurogenesis in the DG (Knoth et al. 2010) and in the densities of dendritic spines in the hippocampus (von Bohlen und Halbach et al. 2006b). In addition, the CNS cholinergic projections undergo moderate degenerative changes during aging (Schliebs and Arendt 2011). To get insight into possible age-related alterations, we therefore decided not only to evaluate in depth the DG-associated morphology in adult p75NTR^{ExIII} deficient mice but we also included aged 20-month-old p75NTR^{ExIII} deficient mice into our analysis.

Materials and methods

Animals

Given the conflicting results reported for p75NTR deficient mice, it is mandatory to compare mutant animals with wild-type mice of the same line (Naumann et al. 2002). For our analysis, we obtained p75NTR^{ExIII} mutant mice (Lee et al. 1992) in their original background from Charles River Laboratories (homozygous and wild-type B6.129S4-

Ngfr^{tm1Jae}/J; stock number: 002213). Homozygous knock-out mice (*p75NTR^{ExIII-/-}*) were analyzed in comparison with age-matched littermate controls (*p75NTR^{ExIII+/+}*) that were obtained by crossing heterozygous *p75NTR^{ExIII}* mice. For the subsequent analysis, mice with an age of 4–6 months (“adult”) as well as with an age of 20 months (“aged”) were used.

Histology

Animals were killed with an overdose of ether and transcardially perfused with phosphate-buffered saline (PBS: 2.0 g NaH₂PO₄, 10.73 g Na₂HPO₄ and 9.0 g NaCl in 1,000 ml distilled water, pH 7.2) followed by perfusion with 4 % paraformaldehyde (PFA; dissolved in PBS). Brains were removed and immersed in the same fixative for several days.

Assessment of layer width and total volume

The width of the granule cell layer and the molecular layer of the DG was measured on a series of 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes, Leiden, The Netherlands; 1:10,000)-stained 30-μm-thick coronal sections sampled at a random start positions. Per section, the width of the upper blade of the granule layer and the molecular layer and of the DG was measured at three different points. A mean of 10 sections was analyzed per animal. The width was calculated as the average across all measured sections. The volume of the DG was determined on serial sections using NeuroLucida (MBF Biosciences, USA).

Determination of cholinergic fiber densities

Coronal sections of 30 μm thickness were made using a vibratome and collected in 20 % ethanol. Free-floating sections were incubated for 18 h with polyclonal goat anti-choline acetyltransferase (ChAT) antiserum (1:100; Millipore, USA) in the presence of 0.1 % Triton-X100. After rinsing, the sections were transferred to biotinylated anti-goat IgG (1:200; Vector, Burlingame, USA) for 2 h at room temperature. After washing, sections were incubated in Cy3-conjugated streptavidin (1:2,000; Jackson Immunoresearch, USA) for 2 h at room temperature. Sections were counterstained with DAPI (1:10,000), washed, and coverslipped in fluorescent mounting medium (DAKO, USA).

Fiber densities were quantified using an Axioplan 2 imaging microscope (Zeiss, Germany). The randomly chosen microscope field of interest (50 × 50 μm) located within either the upper leaf of subgranular layer (SGZ) or the molecular layer of the DG was captured by an Axio-Cam video camera (Zeiss, Germany), connected to a personal computer. A grid consisting of single pixels spaced

2.5 × 2.5 μm apart (in the *x*- by *y*-plane) was overlaid on the image, and fibers intercepting grid points were counted. Relative fiber densities were expressed as $Q = G_i/G_o$. G_i is the number of fibers intercepting the grid points, and G_o is the total number of grid points within the region of interest (von Bohlen und Halbach et al. 2005; von Bohlen und Halbach and Unsicker 2003). At least six different regions of interest were analyzed per zone and animal at different rostro-caudal positions of the DG spaced 120 μm (in the *z*-axis) apart. Data were obtained from six mice per group.

Analysis of adult neurogenesis

The time course of adult hippocampal neurogenesis can be subdivided into different stages during which different markers are expressed (von Bohlen und Halbach 2011). To examine cell proliferation within the DG, phosphohistone H3 was used as a specific marker; Doublecortin (DCX) was used to label newly formed neurons. Coronal sections of 30 μm thickness from the entire hippocampus were cut with a vibratome (Leica VT1000, Germany). The following antibodies and substances were used: rabbit α-phosphohistone H3 (Santa Cruz, Germany), goat α-doublecortin (DCX; 1:200; Santa Cruz, Germany), biotinylated horse α-goat, (1:200; Vector, Germany), biotinylated goat α-rabbit (1:200; Vector, Germany), streptavidin-Cy3 (1:1,000; Jackson Immunoresearch, USA). In case of phosphohistone H3 staining, sections were mounted and air-dried over night. On the next day, sections were washed and then incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave (700 W) for antigen retrieval. After this, sections were incubated in blocking solution (0.1 M PBS, 0.3 % Triton X-100, 3 % serum) for 1 h at RT. In case of DCX immunohistochemistry, we used free-floating sections. Sections were incubated for 1 h in a blocking solution containing 0.3 % Triton X-100 and 3 % bovine serum albumin (BSA) in PBS. Thereafter, sections were incubated in a solution (0.1 Triton X-100 and 3 % BSA in PBS) containing antibodies directed either against phosphohistone H3 or DCX over night at 4 °C. Visualization was done using a biotinylated secondary antibodies Cy3-conjugated streptavidin (Jackson Immunoresearch, USA). Sections were counterstained with DAPI (1:10,000) and washed (the DCX stained sections were mounted and air-dried over night) and then coverslipped in fluorescent mounting medium.

To estimate the number of the labeled newly formed cells (or neurons), cell counts were made using the serial sections. Countings were performed according to the Abercrombie's correction formula. This method renders biases within the range of the optical dissector by taking into account that the particles counted are small compared with the section thickness (von Bartheld 2002). No guard

zones were used for the counting procedure, since we recently have shown that the use of guard zones can bias even optical dissector counting (Baryshnikova et al. 2006). The Linderstrom-Lang/Abercrombie equation for estimating numerical neuronal densities is:

$$N = n \times t/(t + H) \text{ or } N/n = f = t/(t + H)$$

N is an estimate of the number of objects in the defined region, n is the counted number of objects, t is the mean thickness of the virtual section, H is the mean height of the objects, and f is the conversion factor for converting n to N . In a first step, n was counted. In a second step, H , the height of the cells in the z -axis, was estimated using a computer-driven motorized stage (Merzhäuser, Germany) under the control of StereoInvestigator (MBF Biosciences, USA).

Activated caspase-3 staining

For determination of apoptotic cell death, we used antibodies directed against activated caspase-3 (Freund et al. 2013). Coronal sections of 30 μm thickness were mounted and air-dried over night. On the next day, slices were incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave (700 W) for antigen retrieval. After this, sections were washed and then incubated in blocking solution (0.1 M PBS, 0.3 % Triton X-100, 2 % serum) for 1 h at RT. Thereafter, sections were incubated in a solution containing rabbit antibodies (1:250) directed against (active) caspase 3 (AB3623, Millipore, Germany) over night at 4 °C. After washing, sections were incubated in a solution containing Cy3-conjugated goat anti-rabbit IgG (Vector Labs, USA; 1:400, for 1 h). Sections were counterstained with DAPI (1:10,000), washed, and coverslipped in fluorescent mounting medium.

Dendritic spine density

Brains were impregnated according to the Golgi-Cox procedure [using Rapid GolgiStain reagents (FD NeuroTechnologies, USA)] and cut at 120 μm . Analysis of dendritic spines was conducted in a blinded procedure. Only secondary and tertiary dendrites were evaluated, which displayed no breaks in their staining and were not obscured by other neurons or artifacts. Only one segment per individual dendritic branch and neuron was chosen for the analysis. Spines were analyzed in the dorsal, but not ventral, leaf of the DG because spine densities are different in these locations, and no subdivision into different types of spines was made as outlined previously in detail (von Bohlen und Halbach et al. 2006a).

Three-dimensional reconstruction and evaluation were performed using NeuroLucida and a 100× oil immersions

objective, as described previously (Waltereit et al. 2012). Dendrites from granule cells of the DG were used for analysis. At least 20 dendrites per region were reconstructed. The N values for the statistical analysis were based on animal numbers (N) and not on numbers of analyzed elements (n).

Behavioral analyses

Adult male mice (6 months old) were single housed (Macrolon type II cages) with nesting material (paper tissue) in an animal room with constant temperature (23 °C) and adapted to a new 12-h light/dark cycle (lights off from 7 a.m. to 7 p.m.) with free access to food and water. Prior to each test, mice were acclimatized to the experimental room for at least 30 min. Behavioral tests were performed during the dark phase, e.g., in the animals' active phase.

Open field

Before testing, a modified SHIRPA screen was performed for basic assessment of health and neurological function (Rogers et al. 1999). For open-field tests, a quadratic 60 × 60 cm arena was used (Panlab, Spain). Illumination was set to 25 lux. Animals (controls: $n = 15$; p75NTR^{ExIII−/−}: $n = 17$) were placed in the middle of the area and allowed to explore for 5 min. Movements were recorded by a webcam. Parameters characterizing open-field behavior (total distance moved, velocity, resting time, center time, and defecation) were analyzed from recorded sessions using SmartJunior 1.0.0.7 (Panlab). Ethanol 70 % was applied for intertrial cleaning.

Hot plate

Animals (controls: $n = 15$; p75NTR^{ExIII−/−}: $n = 17$) were observed for a maximum of 45 s on a 53 °C (± 0.3 °C) warm hot plate (ATLab, Vendargues, France), where pain nociception (Karl et al. 2003) was interpreted by their latency to lick the hindpaws and/or jump (Chourbaji et al. 2008).

Morris water maze

The Morris water maze provides a comprehensive assay of spatial reference memory (von Bohlen und Halbach et al. 2006b; Vogt et al. 2008).

The animals had to find a platform (14 × 14 cm², Plexiglas) 2 cm under the surface within a water-filled circular pool (diameter: 150 cm) with the help of visual cues attached at the surrounding walls. The water was rendered opaque with 2 l milk kept at 24 °C and renewed daily. Lighting was set to 25 lux. Animals were trained for

4 days with a total of 24 trials (6 trials per day, inter-trial interval min. 1 h) during which the position of the platform was kept unchanged (acquisition phase). In each swim trial during the acquisition phase, mice were left in the pool for a maximum of 120 s or until they found the platform. On the fifth day, the platform was removed and the mouse was swimming for 60 s (probe trial) to assess memory. With a video camera suspended above the center of the pool, the swim tracks of the mice were recorded and analyzed using the image processing system EthoVision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). The following variables from the recorded paths were analyzed: time to find platform (s), length of swim path (m), velocity (cm/s), percent of time spent moving, percent of time spent within a rim of 22 cm from the wall (thigmotaxis), percent of time swimming parallel to the wall. Additional parameters in the probe trial were percent of time in target and other quadrants as well as number of crossings over the former platform position.

Statistics

Student's *t* test and one-way analysis of variance (ANOVA) with Newman–Keuls post hoc analysis were used. Two-way ANOVA with Tukey's multiple comparisons test was used for multiple comparisons when warranted. Data were presented as mean \pm SEM. Significance for all tests was set at $p < 0.05$. Statistical analyses were performed using either SPSS 21 (IBM, USA) or Prism 5.03 (GraphPad Software, USA).

Results

Gross morphology of the dentate gyrus

To get an insight into gross morphological alterations in the DG, we first analyzed the total volume of the DG as well as the width of the granule cell layer and the molecular layer of the upper blade of the DG of p75NTR^{ExIII−/−} mice and in age-matched controls. We found that p75NTR^{ExIII−/−} mutant mice have a slightly bigger DG (+12.8 %) than their corresponding controls (Fig. 1a). Based on that the width of the different layers of the DG was examined in more detail. This examination revealed that the mean width of the granular layer, but not of the molecular layer, was increased (Fig. 1c, e). In addition, we analyzed whether comparable alterations were seen in aged p75NTR^{ExIII−/−} mice as compared to age-matched controls. The aged p75NTR^{ExIII−/−} mice still show a slightly larger volume of the DG as compared to their age-matched controls; however, the difference was not significant (Fig. 1b). Likewise, the thicknesses of the granular and molecular layers were

not different between aged p75NTR^{ExIII−/−} mice and their controls (Fig. 1d, f).

Cholinergic innervation of the dentate gyrus

We next examined whether deletion of p75NTR^{ExIII} has an impact upon the cholinergic system within the DG (Fig. 2). Cholinergic fiber densities in the subgranular and molecular layer of the DG in p75NTR^{ExIII} knockout mice were significantly increased (about 30 %), as compared to age-matched controls. However, in aged animals, the cholinergic fiber densities in both the subgranular and molecular layers did not differ between aged p75NTR^{ExIII} deficient mice and their age-matched controls.

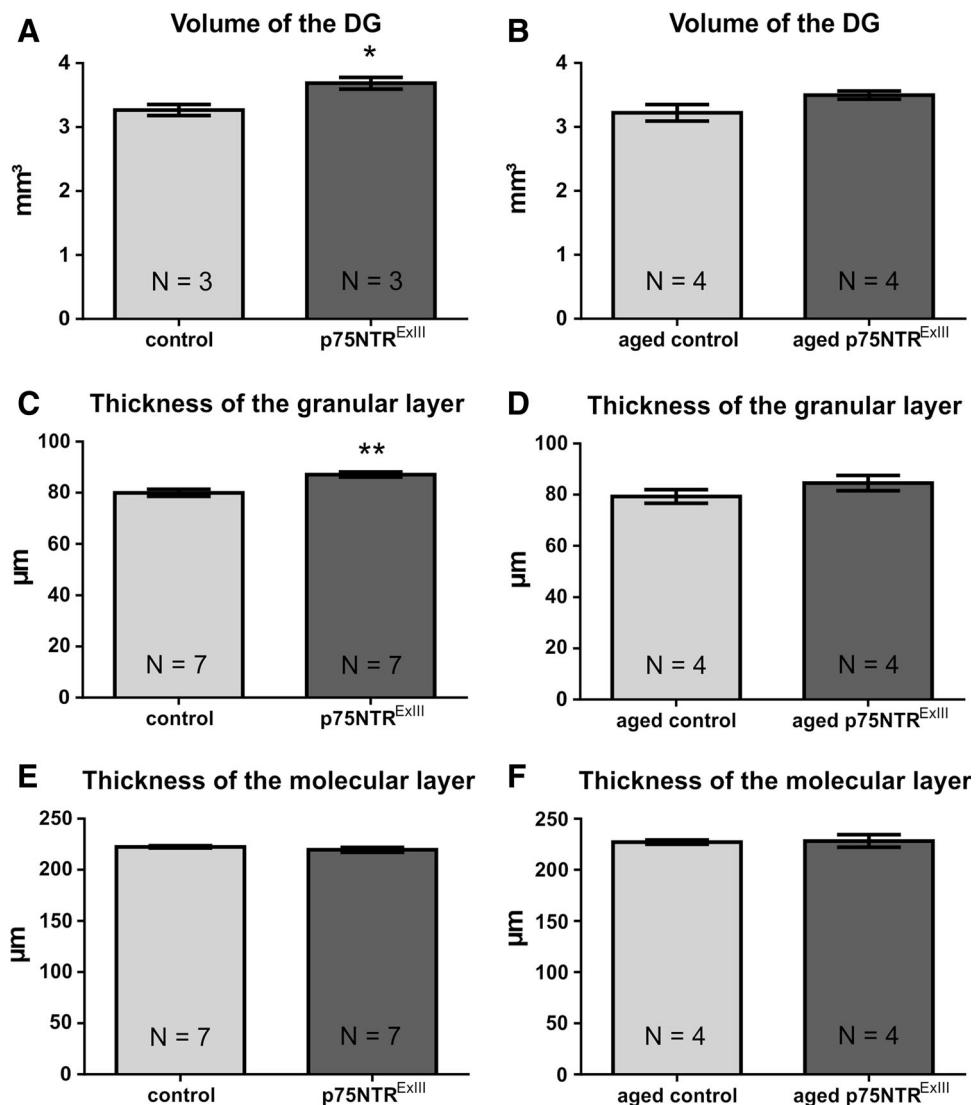
Adult neurogenesis within the dentate gyrus

p75NTR^{ExIII−/−} mice display an increased thickness of the granular cell layer and a higher cholinergic innervation of the SGZ. The higher cholinergic innervation may contribute to increases in adult neurogenesis within the DG (Kaneko et al. 2006). For getting insight into possible alterations in adult neurogenesis, we first determined the effects of a deletion of p75NTR^{ExIII} on cell proliferation within the adult DG. Proliferation was determined by counts of phosphohistone H3 (pH3)-positive cells within the adult DG (Fig. 3a–e). Neither in adult nor in aged p75NTR^{ExIII} deficient mice any differences were found as compared to their controls (Fig. 3e). Thus, cell proliferation in general seems not to be affected by a deletion of p75NTR^{ExIII}. Like BrdU or Ki-67, phosphohistone H3 is a general marker for cell proliferation. These markers do not allow distinguishing between the neuronal and the glial cell lineages. A marker for the neuronal lineage is doublecortin (DCX). DCX is expressed not only by mitotically active young neurons but also by postmitotic young neurons. Therefore, this marker was used for the analysis of the neuronal lineage (Fig. 3g–j). A comparison of adult p75NTR^{ExIII} deficient mice with their respective controls did not reveal a significant difference in the number of DCX-positive cells (Fig. 3f). Aged p75NTR^{ExIII} deficient mice were found to have less DCX-positive cells (264.8 ± 34.5) than age-matched control mice (411.5 ± 38.5); however, this difference was statistically not significant (Two-way ANOVA, followed by Tukey's multiple comparisons test). However, as expected, a significant age-related decrease in DCX-positive cells was revealed for both genotypes, respectively (Fig. 3f).

Cell death within the adult dentate gyrus

It has been reported that neurotrophins induce death of hippocampal neurons via p75NTR receptor signaling (Troy

Fig. 1 Volume and mean thickness of the granular and molecular layer of the DG. **a** The total volume of the DG is increased in p75NTR deficient mice. **b** The total volume of the dentate gyrus of aged p75NTR deficient mice did not differ from the volume of the dentate gyrus of age-matched controls. **c** Thickness of the granular layer of the DG of p75NTR deficient mice and corresponding controls. **d** Thickness of the granular layer of the DG of aged p75NTR deficient mice and age-matched controls. **e** Thickness of the molecular layer of the DG of p75NTR deficient mice and corresponding controls. **f** Thickness of the molecular layer of the DG of aged p75NTR deficient mice and age-matched controls. * $p \leq 0.05$; ** $p \leq 0.001$



et al. 2002; Friedman 2000). Thus, one could expect that p75NTR deficient mice should display reduced rates of cell death within the hippocampus. However, in a study from 2008, p75NTR deficient mice were reported to show increased rates of cell death (Catts et al. 2008). This prompted us to determine the rate of cell death using activated caspase-3 as a marker for apoptosis. As shown in Fig. 3m, the number of apoptotic cells within the DG of p75NTR^{ExIII} deficient mice was lower than in the corresponding control mice.

Dendritic spines of granule cells in the dentate gyrus

Since p75NTR deficiency has been reported to affect dendritic spine densities of CA1 pyramidal neurons (Zagrebelsky et al. 2005), we next investigated whether p75NTR^{ExIII} mice display alterations in dendritic spine densities of granule cells. Figure 4a provides evidence that

densities of dendritic spines were increased in adult p75NTR^{ExIII} mice. However, during aging, dendritic spine densities can be significantly remodeled. In our analysis of DG granule cell dendrites, spine densities in aged (20 months old) p75NTR^{ExIII} mice were comparable to those of aged control mice (Fig. 4b). Concerning the mean length of dendritic spines, no differences were found by comparing adult p75NTR^{ExIII} mice with age-matched controls (Fig. 4c, e, f). Likewise, no alteration in mean spine length was noted by comparing aged p75NTR^{ExIII} mice with age-matched controls (Fig. 4d).

Behavior

The SHIRPA primary screen revealed increased spontaneous activity in knockout animals (control: 1.57 ± 0.147 ; knockout: 2.05 ± 0.185 ; $p \leq 0.05$). On the hind paws, we observed thickened toes and reduced grip strength (control:

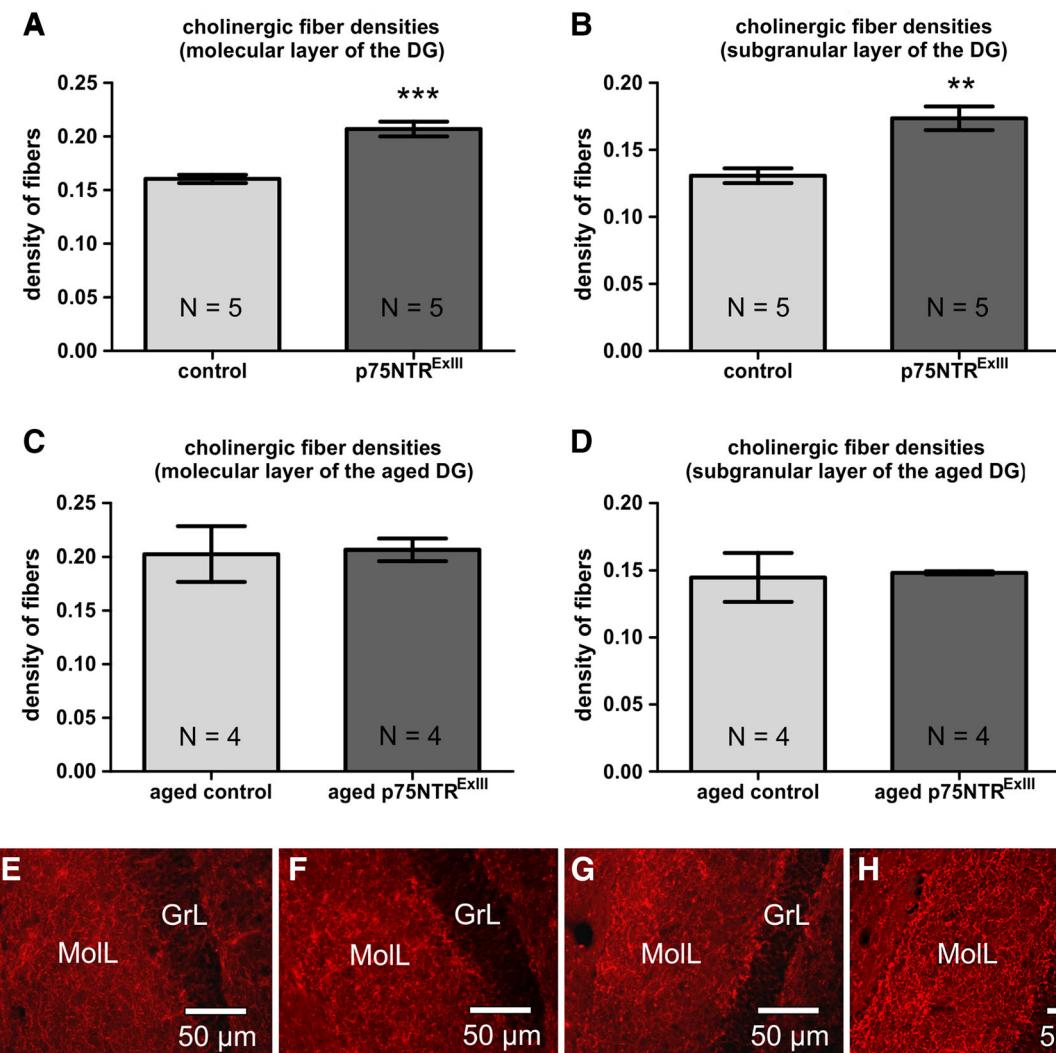


Fig. 2 Densities of cholinergic fibers within the DG are increased in adult p75NTR deficient mice. **a** Cholinergic fiber densities are altered in the molecular layer of adult p75NTR deficient mice. **b** Cholinergic fiber densities in the SGZ are increased in adult p75NTR deficient mice, as compared to age-matched controls. **c** No change in the densities of cholinergic fibers was detected in the molecular layer by comparing aged p75NTR deficient mice and their age-matched controls. **d** No change in the densities of cholinergic fibers was

detected in the subgranular layer by comparing aged p75NTR deficient mice and their age-matched controls. **e** Immunohistological visualization of cholinergic fibers in the DG of adult control mice. **f** Immunohistological visualization of cholinergic fibers in the DG of adult p75NTR deficient mice. **g** Immunohistological visualization of cholinergic fibers in the DG of aged control mice. **h** Immunohistological visualization of cholinergic fibers in the DG of aged p75NTR deficient mice. *Gr* granular layer, *MolL* molecular layer

3.24 ± 0.136 ; knockout: 2.50 ± 0.185 ; $p \leq 0.01$). This might be caused by a deficit in sensory innervation, as previously described (Lee et al. 1992).

In the open-field trial, p75NTR^{ExIII/-} mice displayed increased locomotion (mean distance moved: control: $1,618 \pm 110.1$ cm; knockout: $2,152 \pm 90.89$ cm; $p \leq 0.01$; Fig. 5a) and velocity (control: 5.07 ± 0.35 cm/s; knockout: 6.47 ± 0.33 cm/s; $p \leq 0.01$; Fig. 5b). In addition, the p75NTR^{ExIII/-} mice exhibited reduced resting times (control: $47.03 \pm 2.795\%$; knockout: $37.89 \pm 1.893\%$; $p \leq 0.05$). The number of entries into the center zone of the open field was not different between genotypes

(control: 39.14 ± 3.06 ; knockout: 38.75 ± 2.09 ; $p = 0.92$), but the knockout animals showed a lower rate of defecation ($p \leq 0.01$). Both, knockouts and controls, showed comparable levels of habituation in repeated trials (data not shown).

In the hot plate test, no significant difference was found between the two genotypes.

During the acquisition phase of the Morris water maze test, all mice learned the task demonstrated by reducing latency and swim path to find the platform (time to find platform F(11,242) = 18,039; $p < 0.001$; length of swim path F(11,242) = 29,514; $p < 0.001$); no significant

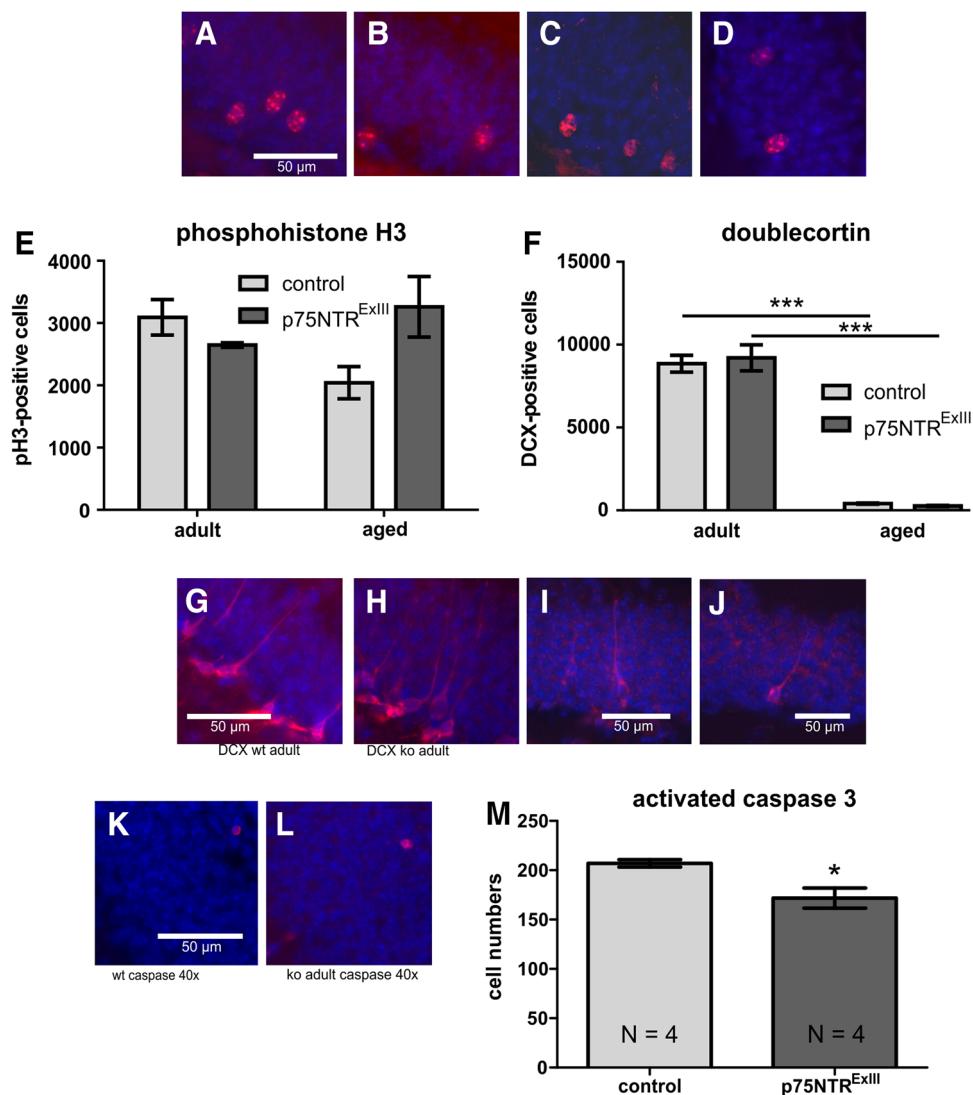


Fig. 3 No major alterations in adult hippocampal neurogenesis of p75NTR deficient mice. **a** Phosphohistone H3 (pH3) immuno-positive cells (red) in the DG of an adult p75NTR^{+/+} mouse. Cell nuclei were visualized with DAPI (blue). **b** Phosphohistone H3 (pH3) immuno-positive cells (red) in the DG of an adult p75NTR^{-/-} mouse. Cell nuclei were visualized with DAPI (blue), same magnification as in (a). **c** Phosphohistone H3 (pH3) immuno-positive cells (red) in the DG of an aged p75NTR^{+/+} mouse. Cell nuclei were visualized with DAPI (blue), same magnification as in (a). **d** Phosphohistone H3 (pH3) immuno-positive cells (red) in the DG of an aged p75NTR^{-/-} mouse. Cell nuclei were visualized with DAPI (blue), same magnification as in (a). **e** No alteration in the number of pH3-positive cells in adult as well as aged p75NTR deficient mice was found as compared to age-matched controls (Two-way ANOVA, followed by Tukey's multiple comparisons test). **f** No alteration in the number of doublecortin-positive cells in adult as well as aged p75NTR deficient mice was found as compared to age-matched controls, but a strong

significant age-related decline in the numbers of DCX-positive cells was detected (Two-way ANOVA, followed by Tukey's multiple comparisons test). **g** Doublecortin (DCX) immuno-positive cells (red) in the DG of an adult p75NTR^{+/+} mouse. Cell nuclei were visualized with DAPI (blue). **h** DCX immuno-positive cells (red) in the DG of an adult p75NTR^{-/-} mouse. Cell nuclei were visualized with DAPI (blue), same magnification as in (g). **i** DCX immuno-positive cells (red) in the DG of an aged p75NTR^{+/+} mouse. Cell nuclei were visualized with DAPI (blue). **j** DCX immuno-positive cells (red) in the DG of an aged p75NTR^{-/-} mouse. Cell nuclei were visualized with DAPI (blue). **k** Example of an activated caspase 3 labeled cell in the granular layer of the DG in an adult p75NTR^{+/+} mouse. **l** Example of an activated caspase 3 labeled cell in the granular layer of the DG in an adult p75NTR^{-/-} mouse. **m** Cell death, as monitored with activated caspase 3, is reduced in p75NTR deficient mice. * $p \leq 0.05$; *** $p \leq 0.001$

alterations were found between genotypes concerning these parameters (Fig. 5c). However, knockout animals swam more slowly ($F(1,22) = 14.749; p = 0.001$), although this did not confound the success to find the platform. In the

probe trial, the p75NTR deficient mice showed significant impairments of memory retrieval: They spent less time in the goal quadrant (controls: 41.5 ± 5.1 s; knockout: 24.9 ± 2.1 s; $p(\text{genotype} \times \text{position}) \leq 0.05$; Fig. 5d) and

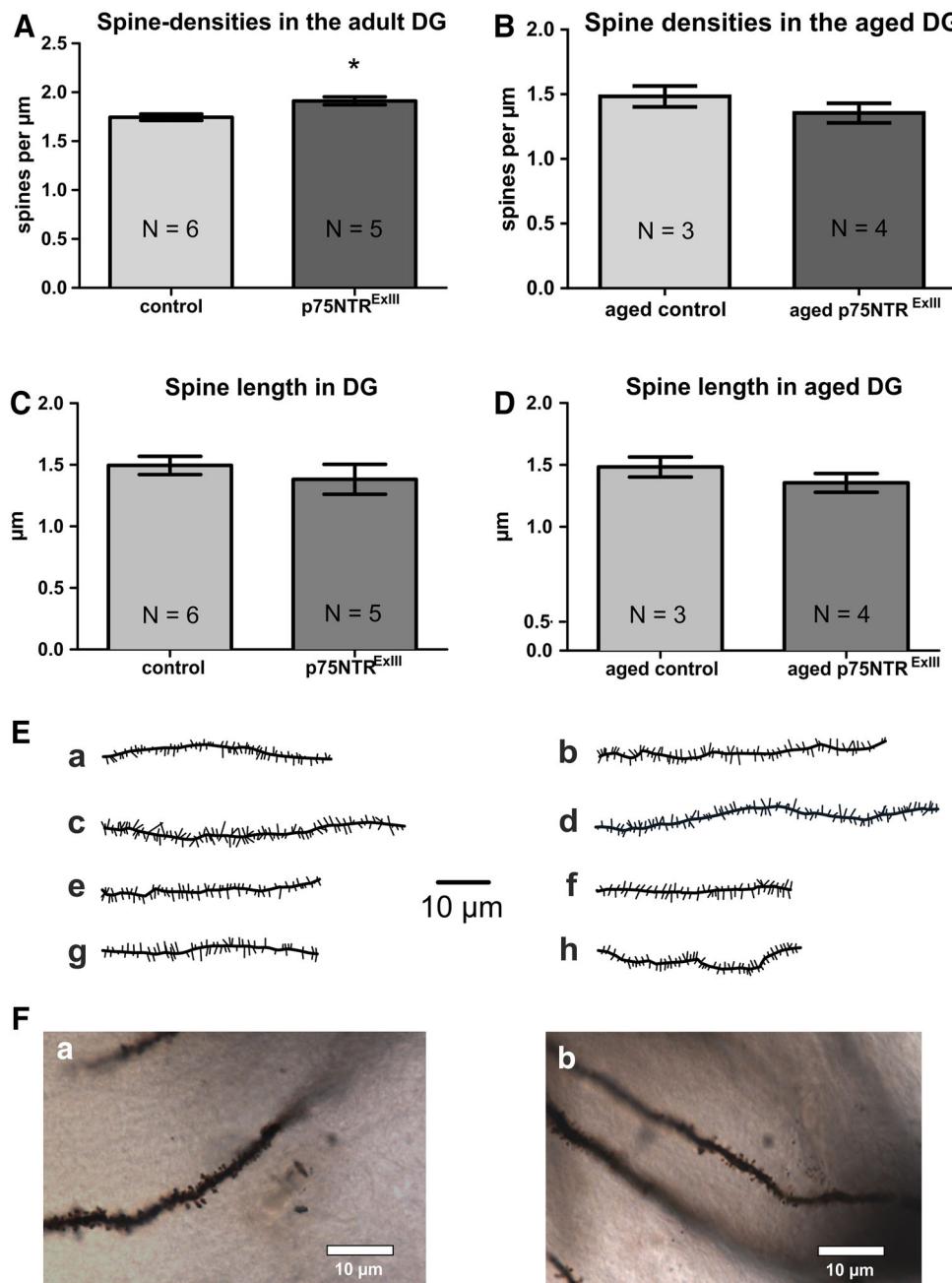
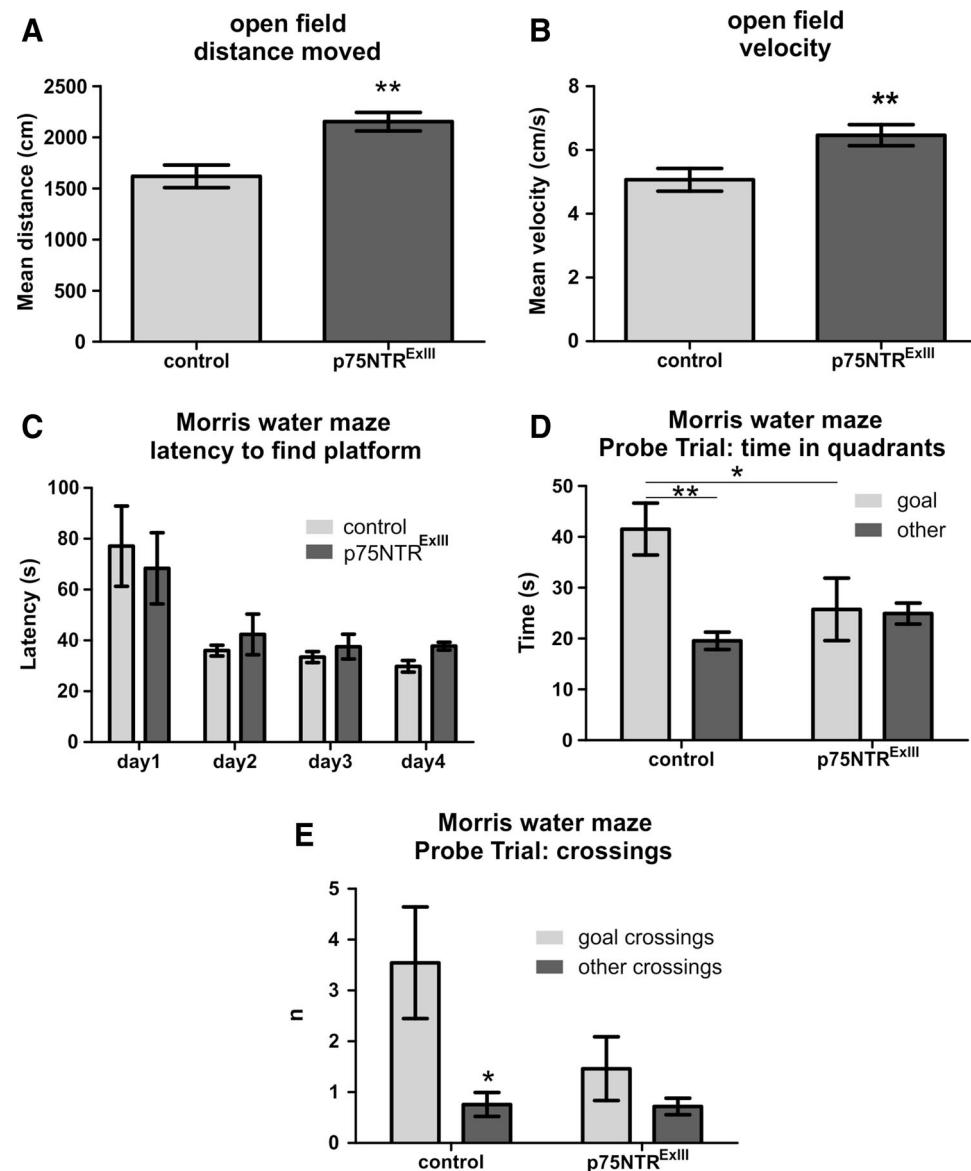


Fig. 4 Spine densities of granule cells of the DG. **a** Spine densities of granule cells of the DG are increased in adult p75NTR deficient mice as compared to their age-matched controls. **b** Spine densities of granule cells of the aged DG are not different between p75NTR deficient mice and age-matched controls. **c** No difference in the mean length of the dendritic spines of DG granule cells was noted by comparing adult p75NTR deficient mice and age-matched controls. **d** The mean spine length of granule cells of the aged DG is not different between p75NTR deficient mice and age-matched controls. **e** Examples of NeuroExplorer (MBF, USA) based visualization of dendritic segments of granule cells located in the DG of adult control (**a** [length: 47.8 μm ; mean spine density: 1.73], **b** [length: 62.4 μm ; mean spine density: 1.60]) and age-matched p75NTR deficient mice

(**c** [length: 61.3 μm ; mean spine density: 1.95], **d** [length: 70.3 μm ; mean spine density: 1.85]) as well as in the DG of aged control (**e** [length: 47.0 μm ; mean spine density: 1.61], **f** [length: 42.6 μm ; mean spine density: 1.50]) and age-matched p75NTR deficient mice (**g** [length: 45.4 μm ; mean spine density: 1.43], **h** [length: 45.9 μm ; mean spine density: 1.74]) that have been reconstructed using NeuroLucida (MBF, USA). **f** Real images of dendrites in the dentate gyrus (**a** adult control; **b** age-matched p75NTR deficient mice) as seen using a 100 \times oil objective (NA: 1.4). Since only a small segment of the dendrite is clearly visible, the dendrites have been reconstructed throughout the three-dimensional space (see section “Materials and methods”). * $p \leq 0.05$

Fig. 5 Behavioral tests: In the open field, the p75NTR deficient mice show a higher activity. Thus, these animals have moved a greater distance than the age-matched controls (a) and they show a higher velocity (b). In the Morris water maze, both p75NTR deficient mice and their age-matched controls improve their latency in finding the platform (c). In the probe trial of the Morris water maze, the control mice spend more time in the goal quadrant (d) and crossed the quadrant bearing the hidden platform more frequently than the p75NTR deficient mice (e). * $p \leq 0.05$; ** $p \leq 0.01$



they crossed the former platform position less often (controls: 3.55 ± 1.1 ; knockout: 1.46 ± 0.63 ; Fig. 5e).

Discussion

A number of strikingly discrepant reports on the role of the p75NTR in the hippocampus, most notably the DG, prompted this study. On the gross morphological level, we found that the volume of the DG is increased in adult but not aged p75NTR^{ExIII} mutant mice. This prompted us to investigate in more detail the cellular mechanisms that may contribute to this phenotype.

Deficiency of p75NTR has been described to affect dendritic spine densities of CA1 pyramidal neurons in vitro (Zagrebelsky et al. 2005). Similarly, in the DG, we

observed that densities of dendritic spines were increased in adult p75NTR^{ExIII/-} mice, indicating that p75NTR has a role as a negative modulator of dendritic spine density in hippocampal neurons, both pyramidal neurons, and granule cells.

The cholinergic projections from the septal area to the hippocampus have been proposed to be important in cognition by modulating properties of the hippocampal network (Teles-Grilo Ruivo and Mellor 2013). Interestingly, up to 75 % increased levels of ChAT activity in the hippocampus were reported for adult p75NTR^{ExIII} knockout compared to wild-type mice (Barrett et al. 2010). This increase may result from a higher cholinergic innervation of the hippocampus, possibly related to an increase in volume, or higher levels of ChAT activity per cholinergic neuron. Our results indicate that there is a strong increase

in the cholinergic fiber densities of the DG in adult p75NTR^{ExIII} deficient mice.

Previous analyses of p75NTR^{ExIII} knockout mice have correlated an increased cholinergic innervation of the hippocampus with higher numbers of cholinergic neurons in the basal forebrain (Greferath et al. 2012; Yeo et al. 1997). With respect to a putative impact of aging on the density of hippocampal cholinergic innervation, we found that it did not significantly differ between aged knockout and wild-type mice suggesting that the difference observed at the age of 4–6 months had been leveled off 14–16 months later. In “middle-aged”, i.e., 16-month-old mice, Barrett et al. (2010) had noted hippocampal ChAT activity still increased by 45 % in p75NTR^{ExIII} knockouts indicating that the decline in ChAT may accelerate with increasing age. However, it should be noted that cholinergic fiber density and ChAT activity may not strictly correlate. There is indirect evidence that increased ChAT activity in p75NTR^{ExIII} knockouts is not due to reduced BDNF signaling, since mice with reduced BDNF expression (BDNF^{+/−}) have decreased ChAT activity (Chourbaji et al. 2004). In addition, it has been shown that there is no difference in NGF levels between p75NTR^{ExIII} deficient mice and controls (Yeo et al. 1997).

In the adult hippocampus, neural progenitor cells can give rise to neurons throughout life, and it is known that neurogenesis within the hippocampus is modulated by various intrinsic and extrinsic factors (Kempermann 2011). Deficits in adult hippocampal neurogenesis correlate with disturbances in spatial learning and memory (Dokter and von Bohlen und Halbach 2012; Ming and Song 2011; Ehninger and Kempermann 2008). Recent reports have suggested that the cholinergic system promotes neurogenesis (Mohapel et al. 2005; Kaneko et al. 2006). NGF, which is thought to act as a key neurotrophic factor for cholinergic septal neurons (Hefti and Will 1987) that innervate the hippocampus, has been shown to promote the survival of newly generated neurons in the rat DG, when continuously infused with NGF (Frielingsdorf et al. 2007). Underlying putative mechanisms may include a role of NGF-p75 signaling in neurogenesis. Indeed, adult neurogenesis within the subventricular zone (SVZ) that replaces neurons in the olfactory bulb throughout life depends on p75NTR signaling (Gascon et al. 2007; Young et al. 2007; Sotthibundhu et al. 2009). In the SVZ, p75NTR is expressed by a population of highly proliferative precursor cells (Young et al. 2007; Giuliani et al. 2004). Furthermore, it has been shown that p75NTR is involved in the proliferation of undifferentiated mouse embryonic stem cells (Moscatelli et al. 2009). Thus, it could be speculated that lack of p75NTR has an impact upon adult neurogenesis within the DG. Along a different line, it has been demonstrated that neurotrophins induce death of hippocampal

neurons via p75NTR (Friedman 2000; Troy et al. 2002). A link between increased cholinergic innervation, p75NTR deficiency, and neurogenesis is conceivable, since activation of the cholinergic system has been shown to promote adult hippocampal neurogenesis (Kaneko et al. 2006). Furthermore, it has recently been shown that proNGF mainly acts through p75NTR and that infusion of proNGF reduces hippocampal neurogenesis (Guo et al. 2013). Thus, it could be speculated that p75NTR knockout mice may display enhanced neurogenesis. Nevertheless, there are also reports indicating that p75NTR deficient mice display reduced adult neurogenesis (Catts et al. 2008; Bernabeu and Longo 2010; Colditz et al. 2010). Comparable to the results shown by Martinowich and colleagues (Martinowich et al. 2012), we were not able to detect a robust decline in adult neurogenesis within the DG of adult p75NTR^{ExIII−/−} deficient mice nor could we detect significant differences in aged p75NTR^{ExIII−/−} deficient animals as compared to age-matched controls. Neurotrophin binding to p75NTR can lead to activation of NFκB and subsequently induce cell survival, whereas activation of JNK and caspases can induce cell death (Charalampopoulos et al. 2012). Our data indicate that in adult p75NTR^{ExIII−/−} deficient mice, the rate of apoptosis within the DG is reduced. A slight but insignificant increase in the number of DCX-positive cells has been found in adult p75NTR^{ExIII−/−} deficient mice, which may be due to the reduced rate of apoptosis.

For the first time, aged p75NTR deficient mice have been examined on the morphological level.

We could confirm that aging has an impact upon adult neurogenesis (Klempin and Kempermann 2007). Thus, we could demonstrate that mice, like humans (Knoth et al. 2010), display a very strong loss of DCX-positive cells in the DG during aging. Furthermore, we could demonstrate that aging does not have a major impact upon spine densities in the DG (von Bohlen und Halbach et al. 2006b). However, we could not detect significant differences between aged control and aged p75NTR^{ExIII−/−} deficient mice, neither concerning spine densities nor concerning mean spine length. This may be attributed to a higher variability in the group of aged mice.

The morphological alterations in the hippocampus of adult p75NTR^{ExIII−/−} deficient mice are accompanied by electrophysiological and behavioral changes. It has been shown that hippocampal long-term depression (LTD) is altered (Rosch et al. 2005), but there are conflicting results concerning hippocampal long-term potentiation (Rosch et al. 2005; Barrett et al. 2010). It is thought that there is a functional requirement of hippocampal CA1 LTD for the consolidation of long-term spatial memory (Ge et al. 2010) and that hippocampal LTD has an impact upon Morris water maze behavior in mice (Dong et al. 2013). To examine

whether the alterations observed in p75NTR^{ExIII−/−} deficient mice have an impact upon behavior, we tested these mice in different behavioral paradigms. Concerning hippocampus-related behavior, the Morris water maze test revealed no significant differences between knockout and control mice during the training phase, but in the probe trial, indicating that p75NTR^{ExIII−/−} deficient mice have impairments of spatial memory retention. This may hint to a specific role of the full-length p75NTR in BDNF mediated learning and memory, since BDNF knockout mice also display generally normal behavior, but reduced spatial learning abilities in the Morris water task (Vigers et al. 2012). Thus, the behavioral data can be interpreted as a functional correlate for the essential structural changes (including among others altered cholinergic innervation and changes in dendritic spines) in the hippocampus of adult p75NTR deficient mice.

The present study has confirmed, corrected, and newly established a number of key parameters of hippocampal structure and function of adult and aged p75NTR deficient mice. The study was conducted on mice with a deficiency of Exon III in the p75NTR gene. A deletion of Exon III prevents expression of the full-length receptor. A deletion of Exon IV, which prevents expression of both the full-length receptor and the shorter p75 isoform, may induce even more severe morphological and functional phenotypes. A thorough analysis of adult p75NTR^{ExIV} knockout mice (von Schack et al. 2001; Naumann et al. 2002) and aged p75NTR^{ExIV} deficient mice might give further important insights. Since p75NTR^{ExIV} knockout mice display severe motor impairments (von Schack et al. 2001), hippocampus-related behavior cannot be examined in detail. Nevertheless, examination on the morphological level may allow getting detailed insight into the role of p75NTR in the hippocampus and, in comparison to the data presented here, may also help to determine the role of the short variant of the p75 receptor.

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Impact of a deletion of the full-length and short isoform of p75NTR on cholinergic innervation and the population of postmitotic doublecortin positive cells in the dentate gyrus

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Analyses of mice carrying a deletion of the pan-neurotrophin receptor p75NTR have allowed identifying p75NTR as an important structural regulator of the hippocampus. Most of the previous analyses were done using p75NTR^{ExIII} knockout mice which still express the short isoform of p75NTR. To scrutinize the role of p75NTR in the hippocampus, we analyzed adult and aged p75NTR^{ExIV} knockout mice, in which both, the short and the full-length isoform are deleted. Deletion of these isoforms induced morphological alterations in the adult dentate gyrus (DG), leading to an increase in the thickness of the molecular and granular layer. Based on these observations, we next determined the morphological substrates that might contribute to this phenotype. The cholinergic innervation of the molecular and granular layer of the DG was found to be significantly increased in the knockout mice. Furthermore, adult neurogenesis in the DG was found to be significantly altered with increased numbers of doublecortin (DCX) positive cells and reduced numbers of apoptotic cells in p75NTR^{ExIV} knockout mice. However, cell proliferation as measured by phosphohistone H3 (PH3) positive cell numbers was not affected. These morphological alterations (number of DCX-positive cells and increased cholinergic fiber densities) as well as reduced cell death in the DG are likely to contribute to the observed thickening of the granular layer in p75NTR^{ExIV} knockout mice. In addition, Sholl-analysis of DCX-positive neurons revealed a higher dendritic complexity and could thus be a possible morphological correlate for the increased thickness of the molecular layer in p75NTR deficient animals. Our data clearly demonstrate that deletion of both, the short and the full-length isoform of p75NTR affects DG morphology, due to alterations of the cholinergic system and an imbalance between neurogenesis and programmed cell death within the subgranular zone.

Keywords: neurotrophin, p75NTR, adult neurogenesis, dendritic spine, cholinergic system

Introduction

The neurotrophins are a family of growth factors that includes brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). Neurotrophins exert many of their specific actions through tyrosine kinase (trk) receptors that bind the neurotrophins with high affinity and, in addition, all neurotrophins can signal through a low-affinity receptor, known as p75NTR (see for review: Chao, 2003). Moreover, p75NTR seems to represent a high-affinity receptor for proNGF (see for review: Underwood and Coulson, 2008). The p75NTR is a transmembrane protein composed of an extracellular domain containing several cysteine-rich motifs, a transmembrane domain, and a non-catalytic intra-cellular domain (Underwood and Coulson, 2008). Two isoforms of the p75NTR exist: a short (s-p75NTR) and a full-length isoform. The full-length isoform is capable of binding neurotrophins, whereas the short isoform lacks the neurotrophin binding site. Although the functions of s-p75NTR are largely unknown, some studies suggest that it is a functional receptor *in vivo* (Fujii and Kunugi, 2009).

In order to analyze the functions and roles of p75NTR in more detail, p75NTR knockout mice have been generated. One knockout mouse line has been created by deleting Exon 3, which encodes parts of the extracellular domain (Lee et al., 1992). The homozygote knockout mice (which will be referred to as p75NTR^{ExIII}) are viable and fertile; however they display several defects in the nervous system, including, among others, deficits in the peripheral sensory nervous system (Lee et al., 1992) and loss of sensory neurons in the dorsal root ganglia (Murray et al., 1999). As a consequence of alternative splicing, p75NTR^{ExIII} knockout mice are hypomorph because they still express s-p75NTR (Nykjaer et al., 2005). Another p75 knockout line was generated by von Schack et al. (2001) carrying a deletion of Exon IV (referred to as p75NTR^{ExIV}). Deletion of Exon IV results in a loss of both, the full-length and the short isoform of p75NTR (von Schack et al., 2001). About 40% of the homozygous p75NTR^{ExIV} knockout mice die during the late fetal or early postnatal period, and surviving mice display impaired motility during the first postnatal weeks because of hind limb ataxia (von Schack et al., 2001).

In the adult brain, p75NTR is expressed, *inter alia*, within the hippocampus (Barrett et al., 2005), including the dentate gyrus (DG). Several studies have assessed the putative impact of p75NTR on hippocampal morphology, neurogenesis and hippocampus-related behavior (Wright et al., 2004; Catts et al., 2008; Bernabeu and Longo, 2010; Colditz et al., 2010). However, all these studies were done on p75NTR^{ExIII} deficient mice and provided contradictory results (summarized e.g., in Dokter et al., 2015). The conflicting reports on hippocampal structure and behavior of p75NTR^{ExIII} deficient mice may be attributed to the application of diverse methods used for analysis as well as to the genetic background of the p75NTR^{ExIII} deficient mice and their controls. In addition to the hippocampal phenotype, cholinergic neuron numbers and innervation density were shown to be altered in p75NTR^{ExIII} knockout mice (Van der Zee et al., 1996; Yeo et al., 1997; Naumann et al., 2002; Neseliler et al.,

2011). Interestingly, in p75NTR^{ExIV} knockout mice the increase in cholinergic neurons was reported to be higher, as compared to p75NTR^{ExIII} knockout mice (Naumann et al., 2002). Moreover, it has been shown that hippocampal CA1 pyramidal neurons cultured from p75NTR^{ExIV} mice display a higher increase in spine density than neurons from p75NTR^{ExIII} mice (Zagrebelsky et al., 2005). Despite these different morphological changes in area CA1, both, p75NTR^{ExIII} and p75NTR^{ExIV} knockout mice show similar impairments in hippocampal long-term depression, whereas long-term potentiation (LTP) in both mutants is unaffected (Rösch et al., 2005).

However, it can be speculated that deletion of p75NTR has also an impact upon the DG, since proNGF can signal via p75NTR and since proNGF seems to be capable of inhibiting adult neurogenesis in the DG (Guo et al., 2013). Concerning adult neurogenesis in the DG, it should also be noted that intraventricular injections of 192-IgG saporin in rat severely reduced hippocampal cholinergic innervation and also reduced the number of doublecortin immunoreactive neurons in the DG (Fréchette et al., 2009). Moreover, there are data hinting that acetylcholine may promote neurogenesis (Mohapel et al., 2005). Thus, deletion of p75NTR may affect both, cholinergic innervation of the hippocampus as well as adult neurogenesis. Since the impact of a knockout of p75NTR^{ExIV} upon the DG has not been examined in detail yet, we were therefore interested to analyze whether p75NTR^{ExIV} knockout mice display an altered morphology of the DG. In detail we were interested to see whether (1) the neurotransmitter supply in the hippocampus is affected, whether (2) spine densities of DG granule cells are altered, and (3) whether changes in adult hippocampal neurogenesis can be observed in p75NTR^{ExIV} mice.

Material and Methods

Animals

p75NTR^{ExIV} knockout mice and control littermates were bred from heterozygous mice. These heterozygous mice were offsprings of the strain generated by von Schack and coworkers, 2001 (von Schack et al., 2001). Homozygous knockout mice (p75NTR^{ExIV/-/-}) were analyzed in comparison to littermate controls (p75NTR^{ExIV+/+/-}) that were both obtained by crossing heterozygous p75NTR^{ExIV} mice.

For the subsequent analysis, mice with an age of 4–6 months (“adult”) as well as with an age between 20 and 23 months (“aged”) were used. All animal experiments were performed in accordance with German animal rights regulations and with permission of the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF) Mecklenburg-Vorpommern, Germany.

Histology

Animals were killed with an overdose of ether and transcardially perfused with phosphate-buffered saline (PBS: 2.0 g NaH₂PO₄, 10.73 g Na₂HPO₄ and 9.0 g NaCl in 1,000 ml distilled water, pH 7.2) followed by perfusion with 4% paraformaldehyde (PFA; dissolved in PBS, pH 7.2). Brains were removed and immersed in the same fixative for at least 5 days.

Assessment of Layer Width of the DG

The width of the granule cell layer and the molecular layer of the DG was measured on a series of 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes, Leiden, Netherlands; 1:10,000) stained 30 μm thick coronal serial sections, sampled at a random start position [around Bregma -1.34 mm, determined using a mouse brain atlas (Paxinos and Franklin, 2001)]. Per section the width of the upper blade of the granule layer and the molecular layer and of the DG was measured at three different points. A mean of 10 sections was analyzed per animal (thus 30 measurements per region and animal). The width was calculated as the average across all measured sections using NeuroLucida (MBF Biosciences, USA).

HPLC

From homozygous knockout mice and respective littermate controls brains were removed and frozen in isopentane/dry ice. From 120 μm thick frozen brain sections obtained by using a cryostat (Leica, Nußloch, Germany), tissue samples from hippocampus were collected using specific punching needles. For quantification of monoaminergic neurotransmitters and their metabolites, tissue samples were homogenized in an extraction solution (0.1 M perchloric acid, 1 mM EDTA) using the tissue homogenizer Mixer Mill (Qiagen, Hilden, Germany) and yielded homogenates centrifuged at 15,000 g for 10 min at 4°C. 10 μl of supernatant was applied on a HPLC system with electrochemical detection, consisting of an Antec LC-100 isocratic pump (Shimadzu, Duisburg, Germany), a Spark Triathlon autosampler (Spark Holland, Emmen, The Netherlands), a C18-OptiAqua reverse phase column (150 \times 2.1 mm; 3 μm particle size) (Techlab, Braunschweig, Germany) and a Decade II electrochemical detector (Antec Leyden, Zoeterwoude, The Netherlands). The mobile phase contained 50 mM sodium citrate, 2.1 mM octyl sodium sulfate, 0.1 mM EDTA, 10 mM NaCl, and 23% methanol at pH 4.0. The temperature applied on the system for optimal peak separation was 37°C. Using this protocol, concentrations of dopamine (DA), noradrenaline (NA), serotonin (5-HT) 3, 4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) can be quantified simultaneously by determining the area under each peak using the software Clarity (Data Apex, Prague, Czech Republic) and referencing it to the appropriate standard curve. All tissue concentrations were calculated by normalizing the quantified neurotransmitter amounts to the respective weight of the tissue sample.

Determination of Cholinergic Fiber Densities

30 μm thick coronal sections were made using a vibratome and collected in 20% ethanol. On the next day, slices were incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave (700 W) for antigen retrieval. Thereafter, sections were incubated for 72 h with polyclonal goat anti choline-acetyltransferase (ChAT) antiserum (1:200; Millipore, USA) in the presence of 0.1% Triton-X100. After rinsing the sections were transferred to biotinylated anti-goat IgG (1:200; Vector, Burlingame, USA) for 2 h at room temperature. After washing

sections were incubated in Cy3-conjugated streptavidin (1:2,000; Jackson Immunoresearch, USA) for 2 h at room temperature. Sections were counterstained with DAPI (1:10,000), washed, and coverslipped in fluorescent mounting medium (DAKO, USA).

Fiber densities were quantified using an AxioPlan 2 imaging microscope (Zeiss, Germany). The randomly chosen microscope field of interest (ROI: 50 \times 50 μm) either located within the upper leaf of the subgranular layer (SGZ) or the molecular layer of the DG was captured by an AxioCam video camera (Zeiss, Germany), connected to a personal computer. A grid consisting of single pixels spaced 2.5 \times 2.5 μm apart (in the x- by y-plane) was overlaid on the image and fibers intercepting grid points were counted. Relative fiber densities were expressed as $Q = G_i/G_0$. G_i is the number of fibers intercepting the grid points and G_0 is the total number of grid points within the region of interest (von Bohlen und Halbach, 2013). At least six different regions of interest (starting at around Bregma -1.46 mm) were analyzed per zone and animal at different rostro-caudal positions of the DG, spaced 120 μm (in the z-axis) apart.

Activated Caspase-3 Staining

For determination of apoptotic cell death, we used antibodies directed against activated caspase-3 (Freund et al., 2014). Coronal sections of 30 μm thickness were mounted and air-dried over night. On the next day, slices were incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave (700 W) for antigen retrieval. After this, sections were washed and incubated in blocking solution (0.1 M PBS, 0.3% Triton X-100, 2% serum) for 1 h at RT. Thereafter, sections were incubated in a solution containing rabbit antibodies (1:250) directed against (active) caspase 3 (AB3623, Millipore, Germany) over night at 4°C. After washing, sections were incubated in a solution containing Cy3-conjugated goat anti-rabbit IgG (Vector Labs, USA; 1:400, for 1 h). Sections were counterstained with DAPI (1:10,000), washed and coverslipped in fluorescent mounting medium.

Adult Neurogenesis

The time-course of adult hippocampal neurogenesis can be subdivided into different stages, during which different markers are expressed (von Bohlen und Halbach, 2011). To examine cell proliferation within the DG, phosphohistone H3 was used as a specific marker; NeuroD was used to label mitotic active neuronal cells, and doublecortin (DCX) was used to label newly formed neurons. Coronal sections of 30 μm thickness (or 60 μm in case for Sholl-analysis) from the entire hippocampus were cut with a vibratome (Leica VT1000, Germany). The following antibodies and substances were used: rabbit α -phosphohistone H3 (Santa Cruz, Germany), goat α -NeuroD (1:100; Santa Cruz, Germany), goat α -doublecortin (DCX; 1:200; Santa Cruz, Germany), biotinylated horse α -goat, (1:200; Vector, Germany), biotinylated goat α -rabbit (1:200; Vector, Germany); streptavidin-Cy3 (1:1,000; Jackson Immunoresearch, USA).

For phosphohistone H3 staining, sections were mounted and air-dried over night. On the next day, sections were washed and then incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave oven (700 W) for antigen retrieval. After this, sections were washed in a solution containing 0.1 M PBS,

0.3% Triton X-100, 3% serum) for 1 h at RT. For NeuroD staining, sections were mounted and air-dried. Sections were then washed and then incubated in sodium citrate buffer (pH 6.0) for 20 min using a microwave (700 W) for antigen retrieval. After this, sections were incubated in blocking solution (0.1 M PBS, 0.1% Triton X-100, 1% serum) for 1 h at RT. For DCX immunohistochemistry, we used free-floating sections. Sections were incubated for 1 h in a blocking solution containing 0.3% Triton X-100 and 3% bovine serum albumin (BSA) in PBS.

Thereafter, sections were incubated in a solution (0.1 Triton X-100 and 3% serum in PBS) containing antibodies directed either against phosphohistone H3, NeuroD or DCX over night at 4°C. Visualization was done using a biotinylated secondary Cy3-conjugated streptavidin antibody (Jackson Immunoresearch, USA). Sections were counterstained with DAPI (1:10,000) and washed (the DCX stained sections were mounted and air-dried over night) and then coverslipped in fluorescent mounting medium.

Counting of Labeled Cells

To estimate the number of the labeled cells, cell counts were made using the serial sections, as described previously (Dokter et al., 2015). In brief: countings were performed according to the Abercrombie's correction formula (starting around Bregma –1.06 mm), since this method renders biases within the range of the optical disector by taking into account that the particles counted are small compared with the section thickness (von Bartheld, 2002). No guard zones were used, since the use of guard zones can bias even optical disector counting (Baryshnikova et al., 2006). The Linderstrom-Lang/Abercrombie (LLA) equation for estimating numerical neuronal densities is:

$$N = n * t(t + H) \text{ or } N/n = f = t / (t + H)$$

N is an estimate of the number of objects in the defined region, n is the counted number of objects, t is the mean thickness of the virtual section, H is the mean height of the objects, and f is the conversion factor for converting n to N .

In a first step, n was quantified using an Axioplan 2 imaging microscope (Zeiss, Germany) fitted for fluorescence. In a second step, H , the height of the cells in the z-axis, was estimated using a computer-driven motorized stage (Merzhausen, Germany) connected to the Axioplan 2 imaging microscope (Zeiss, Germany) under the control of StereoInvestigator (MBF Biosciences, USA).

Sholl-Analysis

For identification and reconstruction of DCX-stained neurons, z-stacks (step-width: 1 μm) were generated. From these z-stacks DCX-positive neurons within the granular layer of the DG were reconstructed using NeuroLucida (MBF Biosciences, USA) and the reconstructions were analyzed with the help of NeuroExplorer (MBF Biosciences, USA) using the module "Sholl analysis" for the analysis of the three-dimensional vector-based Neurolucida data sets. Sholl analysis was performed using 6 p75NTR^{ExIV/+} and 5 p75NTR^{ExIV/-} animals. Per animal, a mean of 10 individual neurons were reconstructed. For

visualization of the z-stacks, images were loaded in ImageJ (NIH, USA) and processed using the z-project plug-in (parameter: "Max Intensity").

Analysis of Dendritic Spines

Brains were impregnated according to the Golgi-Cox procedure [using Rapid GolgiStain reagents (FD NeuroTechnologies, USA)] and cut at 120 μm. Analysis of dendritic spines was conducted in a blinded procedure. Only one segment per individual dendritic branch and neuron was chosen for the analysis. Spines were analyzed in the dorsal, but not ventral, leaf of the DG because spine densities are different in these locations and no subdivision into different types of spines [filopodia, stubby, thin, and mushroom, which reflect temporal snapshots of a dynamic phenomenon (Parnass et al., 2000)] was made as outlined previously in detail (von Bohlen und Halbach et al., 2006). Analyses were conducted on Golgi-impregnated sections that were uniformly dark throughout the section. Only dendrites that displayed no breaks in their staining (Leuner et al., 2003) and that were not obscured by other neurons or artifacts (Liu et al., 2001) were evaluated. Three-dimensional reconstruction and evaluation were performed using NeuroLucida and a 100x oil immersions objective, as described previously (Waltereit et al., 2012). Dendrites from granule cells of the DG were used for analysis. At least 30 dendrites per region and animal (n) were reconstructed with approximately 3000 individual spines per animal. The n values for the statistical analysis were based on animal numbers and not on numbers of analyzed elements.

Statistics

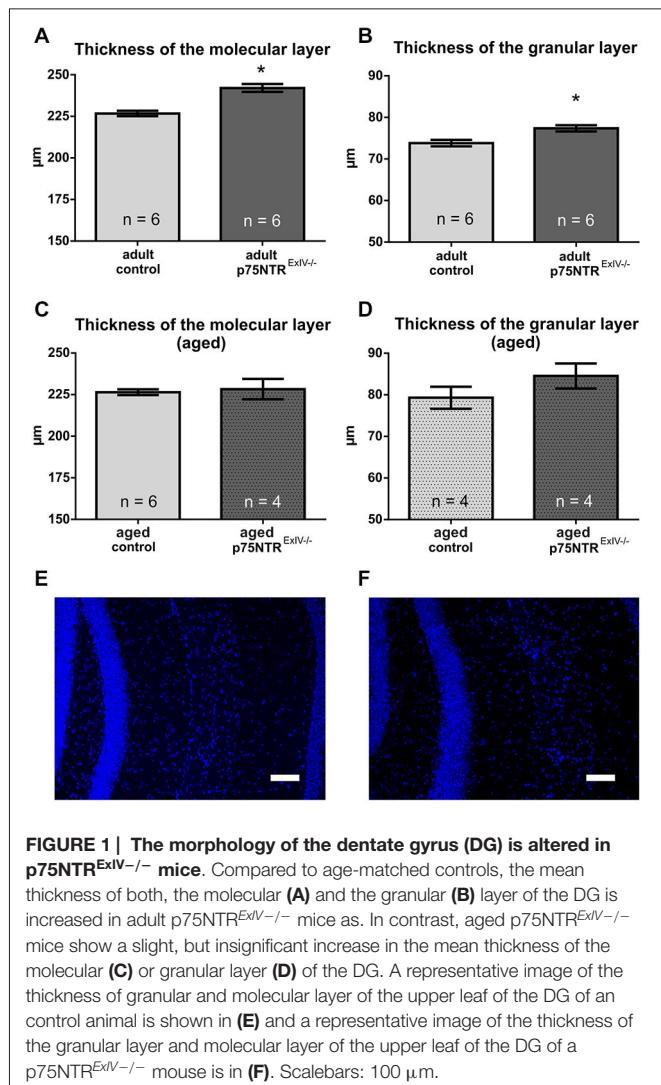
Statistical analyses were performed using Prism 6.0 for Windows (GraphPad Software Inc., USA). For statistical evaluation of two groups unpaired two-sided t -tests were used.

For the analysis of more than two groups one-way ANOVA followed by a Tukey's multiple comparisons test was used. In case of the HPLC analysis, a two-way ANOVA followed by a Bonferroni post-test was used. Significance levels for all tests were set at $p \leq 0.05$. Data in figures were expressed as mean ± SEM; significant changes in the figures were indicated by an asterisk.

Results

Thickness of the Molecular and Granular Layer of the DG

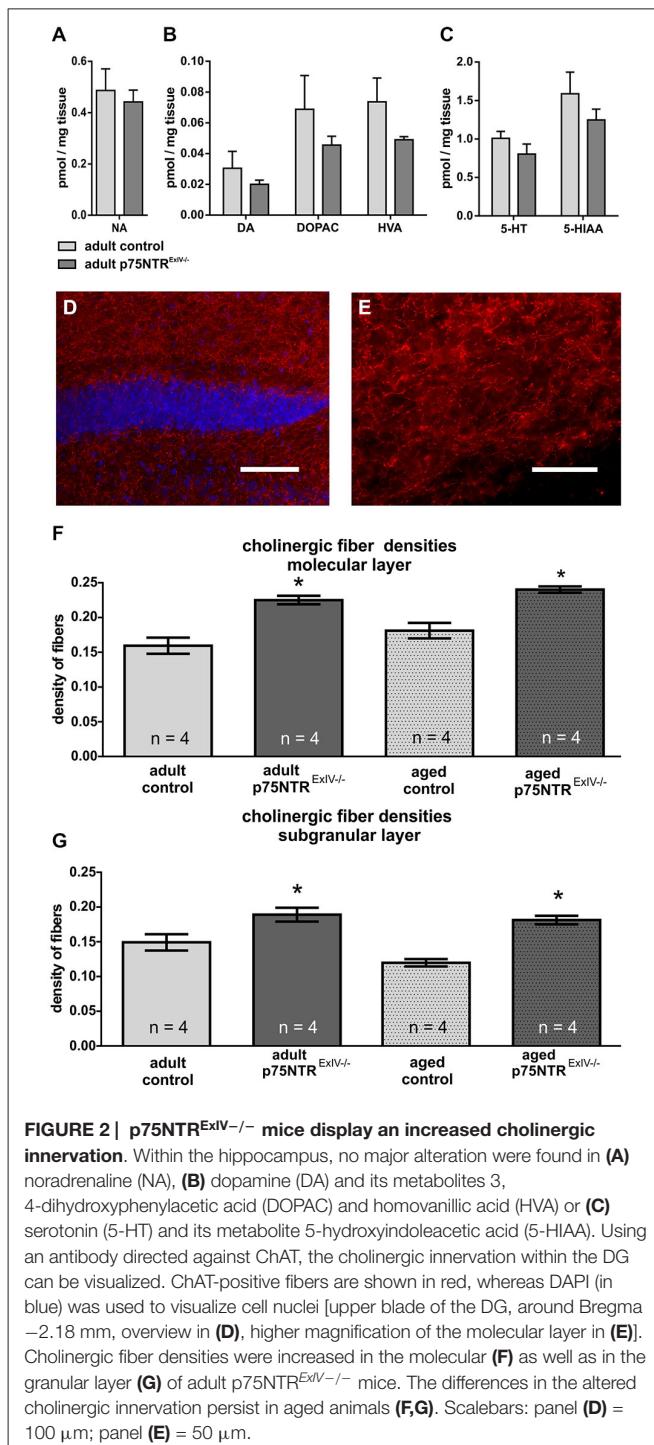
We have previously shown that functional deletion of the long isoform of the p75 receptor (p75NTR^{ExIII} knockout) causes a significant increase of the DG resulting from an increased width of the granular, but not the molecular layer in adults (Dokter et al., 2015). Here, we show that deletion of both, the long and the short isoform of the p75 receptor leads to an increase in thickness of the molecular (6.8%; $p = 0.0004$) as well as the granular layer (4.8%; $p = 0.008$) in adult p75NTR^{ExIV} knockout mice (Figure 1). Thus, deletion of both, the long and the short isoform of the p75 receptors aggravates the phenotype concerning DG morphology. However, no apparent differences were observed in aged animals, (Figures 1C,D), neither in the granular layer ($p = 0.240$) nor in the molecular layer ($p = 0.735$).



Cholinergic Innervation of the DG

We first analyzed the integrity of dopaminergic, noradrenergic or serotonergic systems within the hippocampus of *p75NTR^{ExIV-/-}* mice. Surprisingly, no major alterations concerning tissue concentrations and metabolism of the monoaminergic neurotransmitters such as NA (Figure 2A), DA (Figure 2B), and serotonin (Figure 2C) were found (for statistics, see Table 1).

We next examined whether and to what extent deletion of *p75NTR^{ExIV}* may affect the cholinergic system within the DG. In Figure 2 the data concerning altered cholinergic innervation in adult and aged *p75NTR^{ExIV}* are summarized. Our results indicate that cholinergic fiber densities in the molecular ($p = 0.002$) and subgranular ($p = 0.041$) layer of the DG in *p75NTR^{ExIV}* knockout mice were significantly increased by more than 32%, as compared with age-matched controls (Figure 2F). Thus, deletion of the short, in addition to the long receptor isoform, does not increase cholinergic hyperinnervation beyond the level seen in mice with a deletion of the long receptor isoform only (+30%; cf.; Dokter et al., 2015). The increase in cholinergic fiber density persisted in aged *p75NTR^{ExIV-/-}* mice (Figure 2F), in contrast



to the *p75NTR^{ExIII}* knockout mice (Dokter et al., 2015), in the molecular ($p = 0.003$) as well as subgranular layer ($p = 0.0003$) of the DG.

Cell Death within the Adult Dentate Gyrus

We have recently shown that the rates of cell death in the hippocampus of *p75NTR^{ExIII}* knockout mice are reduced as compared to controls (Dokter et al., 2015). Using activated

TABLE 1 | Statistical analysis of the data obtained by the HPLC measurements.

control	P75NTR ^{ExIV−/−}	Difference	t	P value
NA	0.4867	0.4419	-0.04475	0.2997 $P > 0.05$
Dopamin	0.03048	0.02000	-0.01048	0.07015 $P > 0.05$
DOPAC	0.06881	0.04548	-0.02333	0.1562 $P > 0.05$
HVA	0.07361	0.04896	-0.02465	0.1650 $P > 0.05$
5-HIAA	1.588	1.248	-0.3401	2.278 $P > 0.05$
5-HT	1.009	0.8030	-0.2060	1.380 $P > 0.05$

Bonferroni's multiple comparisons test p75NTR^{ExIV−/−} vs. control (HPLC).

Abbreviations: 5-HIAA = 5-hydroxyindoleacetic acid; 5-HT = serotonin; DA = dopamine; DOPAC = 3, 4-dihydroxyphenylacetic acid, HVA = homovanillic acid; NA = noradrenaline.

caspase-3 as a marker for apoptosis; **Figures 3A–C**) we found that p75NTR^{ExIV} knockout mice display a slightly stronger reduction of apoptotic cell death in the DG as compared to age-matched controls (**Figure 3A**; $p = 0.013$). Aged p75NTR^{ExIV−/−} mice also showed reduced rates of apoptosis, but due to the great variance observed in older animals, the difference was not significant from age-matched controls (**Figure 3B**; $p = 0.484$).

Adult Neurogenesis within the Dentate Gyrus

To further assess possible alterations in adult neurogenesis, we first studied the effects of a deletion of both, the truncated and long isoform of p75NTR on cell proliferation within the adult DG using phosphohistone H3 (PH3) as a marker (**Figure 3D**). In adult mice, the number of PH3 positive cells within the DG of p75NTR^{ExIV−/−} mice was not different from the number determined in control mice ($n = 4$ per group; $p \leq 0.58$; **Figure 3E**). Likewise, aged p75NTR^{ExIV−/−} showed no alteration in the number of PH3 positive cells as compared to age-matched controls ($n = 4$ per group; $p \leq 0.12$). Thus, neither deletion of Exon III (Dokter et al., 2015) nor of Exon IV of the p75NTR gene affects cell proliferation within the DG.

We next analyzed alterations within the early neuronal lineage by using doublecortin (DCX) as a marker for both mitotically active and postmitotic young neurons (**Figure 3F**). As shown in **Figure 3G**, adult p75NTR^{ExIV−/−} mice harbor significantly more DCX positive cells within the DG than age-matched p75NTR^{ExIV+/+} mice ($n = 6$; $p = 0.01$). This is in contrast to mice with a deletion of Exon III (Dokter et al., 2015).

In line with previous reports (Kuhn et al., 1996; Klempin and Kempermann, 2007) neurogenesis declines with age (**Figure 3G**). Similar to p75NTR^{ExIII−/−} mice (Dokter et al., 2015), aged p75NTR^{ExIV−/−} mice did not display significant alterations in the number of DCX-positive cells as compared with age-matched controls ($n = 3$; **Table 2**). The overall increase in the population of DCX-positive cells in adult mice prompted us to analyze this effect in more detail.

First, the number of mitotically active young neurons was determined using NeuroD (von Bohlen und Halbach, 2007). As shown in **Figure 4A**, the number of NeuroD positive cells within the DG of p75NTR^{ExIV−/−} mice (2205 ± 259.8 ; $n = 6$) was not statistically different (*t*-test: $p = 0.83$) from the numbers

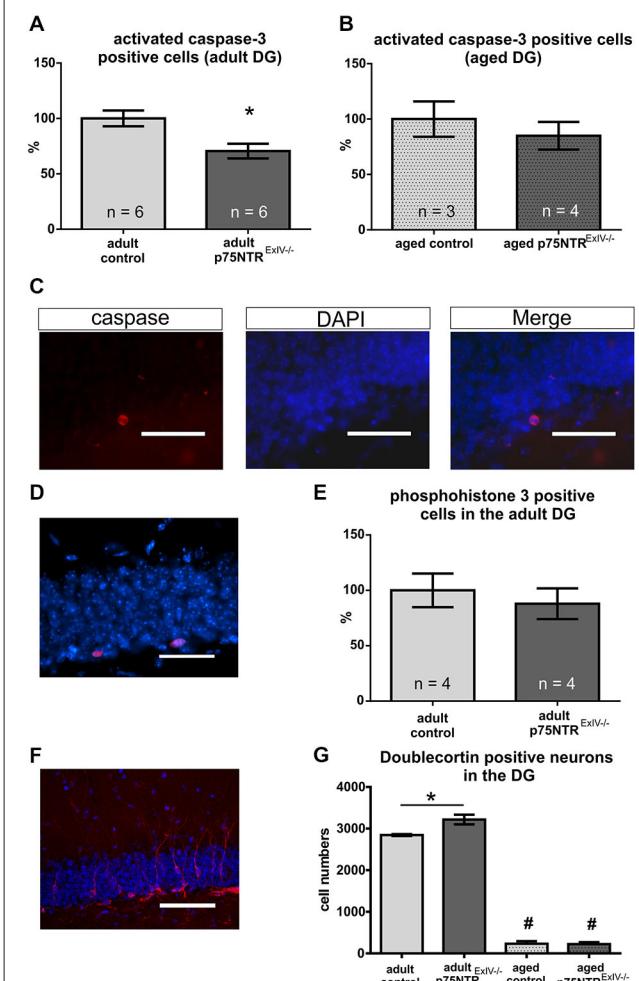


FIGURE 3 | Impact of a deletion of p75NTR on apoptosis, proliferation and differentiation. Apoptotic cell death was monitored using caspase 3.

Adult p75NTR^{ExIV−/−} mice display a strong reduction in the rate of apoptosis in the DG (**A**). Aged p75NTR^{ExIV−/−} mice still display reduced rates of apoptosis; however, not significantly different from aged controls (**B**). In (**C**) an example of a caspase 3 immuno-positive cell within the DG is displayed. Cell proliferation in the adult DG was analyzed using phosphohistone H3 (PH3) immunohistochemistry [**D**; PH3 positive cells in red; DAPI (in blue was used to visualize cell nuclei)]. No significant alteration in the number of PH3-positive cells could be determined by comparing adult p75NTR^{ExIV−/−} and age-matched controls (**E**). For analysis of the neuronal lineage doublecortin (DCX) was used as a specific marker (**F**; DCX positive neuron are shown in red; DAPI (in blue) was used to visualize cell nuclei). During aging, there is a dramatic reduction in the number of DCX-positive cells (**G**) as compared to adult animals of the same genotype (significant changes are indicated by #). Adult p75NTR^{ExIV−/−} mice have significant more DCX positive neuronal cells than age-matched controls (One-way ANOVA followed by a Tukey's multiple comparisons test). Scalebars: panel (**C,D**): 50 μm; panel (**E**): 100 μm.

determined in the respective controls (2136 ± 165 ; $n = 6$; **Figure 4B**).

Since the number of NeuroD positive cells remained unchanged, we next asked whether the observed phenotype regarding the increase in DCX positive cells might be attributable to an increase in the number of postmitotic young DCX-positive

TABLE 2 | Statistical data from the analysis of doublecortin (DCX) positive neurons within the DG.

	Mean Diff.	95% CI of diff.	Significant?		
control vs. p75NTR ^{ExIV−/−}	−373.7	−669.6 to −77.84	Yes (*)		
control vs. aged control	2613	2250 to 2975	Yes (**)		
p75NTR ^{ExIV−/−} vs. aged p75NTR ^{ExIV−/−}	2994	2632 to 3357	Yes (**)		
aged control vs. aged p75NTR ^{ExIV−/−}	8.000	−410.4 to 426.4	No		
Test details	Mean 1	Mean 2	Mean diff.	q	DF
control vs. p75NTR ^{ExIV−/−}	2845	3218	−373.7	5.192	14
control vs. aged control	2845	232.0	2613	29.64	14
p75NTR ^{ExIV−/−} vs. aged p75NTR ^{ExIV−/−}	3218	224.0	2994	33.97	14
aged control vs. aged p75NTR ^{ExIV−/−}	232.0	224.0	8.000	0.0786	14

Tukey's multiple comparisons test p75NTR^{ExIV−/−} vs. control (Doublecortin).

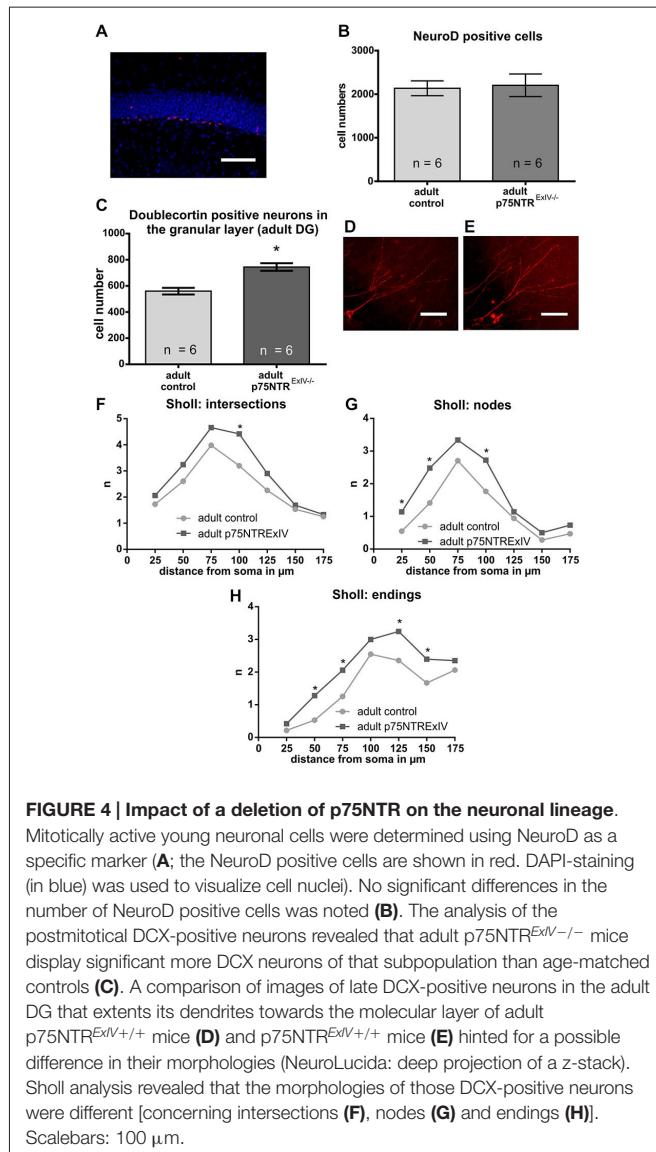


FIGURE 4 | Impact of a deletion of p75NTR on the neuronal lineage. Mitotically active young neuronal cells were determined using NeuroD as a specific marker (**A**; the NeuroD positive cells are shown in red. DAPI-staining (in blue) was used to visualize cell nuclei). No significant differences in the number of NeuroD positive cells was noted (**B**). The analysis of the postmitotical DCX-positive neurons revealed that adult p75NTR^{ExIV−/−} mice display significant more DCX neurons of that subpopulation than age-matched controls (**C**). A comparison of images of late DCX-positive neurons in the adult DG that extends its dendrites towards the molecular layer of adult p75NTR^{ExIV+/+} mice (**D**) and p75NTR^{ExIV+/+} mice (**E**) hinted for a possible difference in their morphologies (NeuroLucida: deep projection of a z-stack). Sholl analysis revealed that the morphologies of those DCX-positive neurons were different [concerning intersections (**F**), nodes (**G**) and endings (**H**)]. Scalebars: 100 μm.

neurons. To estimate the number of those cells, only DCX positive cells were counted that are located within the granular layer of the DG and extend their DCX-positive dendrites towards the molecular layer of the DG. Our analysis revealed that p75NTR^{ExIV−/−} mice have significantly more postmitotic DCX positive young neurons than p75NTR^{ExIV+/+} mice (p75NTR^{ExIV−/−}: 744.7 ± 29.14; n = 6; p75NTR^{ExIV+/+}: 560.1 ± 25.17; n = 6; p = 0.0007; **Figure 4C**). Thus, we assume that deletion of both, the truncated and full-length isoform of p75NTR affects the differentiation of newly generated neurons. Since DCX-positive neurons of p75NTR^{ExIV+/+} mice (**Figure 4D**) seemed to have different morphologies as compared with DCX-positive neurons of p75NTR^{ExIV−/−} mice (**Figure 4E**), Sholl-analyses were conducted. Indeed, as shown in **Figures 4F–H**, DCX-positive neurons of the two genotypes displayed different morphologies, which was apparent in the number of dendritic intersections, nodes and endings. Thus, the morphologies of DCX positive neurons of p75NTR^{ExIV−/−} mice appear to be more complex than DCX positive neurons of control animals (see for statistics: **Table 3**).

Dendritic Spines of Granule Cells in the Dentate Gyrus

Since p75NTR deficiency has been reported to affect dendritic spine densities of CA1 pyramidal neurons in organotypic cell cultures derived from p75NTR^{ExIII−/−} as well as p75NTR^{ExIV−/−} mice (Zagrebelsky et al., 2005), we next investigated whether p75NTR^{ExIV−/−} mice display alterations in dendritic spine densities of mature granule cells. Adult p75NTR^{ExIV−/−} mice, in contrast to adult p75NTR^{ExIII−/−} mice (Dokter et al., 2015), display similar spine densities as age-matched controls, whereas in aged p75NTR^{ExIV−/−} as well as p75NTR^{ExIII−/−} (Dokter et al., 2015) knockout animals no impact on spine densities within the DG was found. Furthermore, in both, p75NTR^{ExIV−/−} mice and age-matched controls, an age-related decline in spine densities was observed (**Figure 5A**). Thus, in controls, the spine densities declines significantly (p = 0.007) during aging from 17.33 to 14.81 spines per 10 μm and in p75NTR^{ExIV−/−} mice an age-related

TABLE 3 | Summary of the statistical differences (Sholl-analysis).

Distance from soma (μm)	Nodes	Intersection	Ending
25	0.0016	0.0760	0.0954
50	0.0013	0.0689	0.0012
75	0.0637	0.0613	0.0106
100	0.0052	0.0022	0.2494
125	0.4572	0.1856	0.0065
150	0.2396	0.7658	0.0295
175	0.5393	0.9084	0.5276
200	0.3019	0.4108	0.5586

Sholl-analysis (*t*-test: *p*-values).

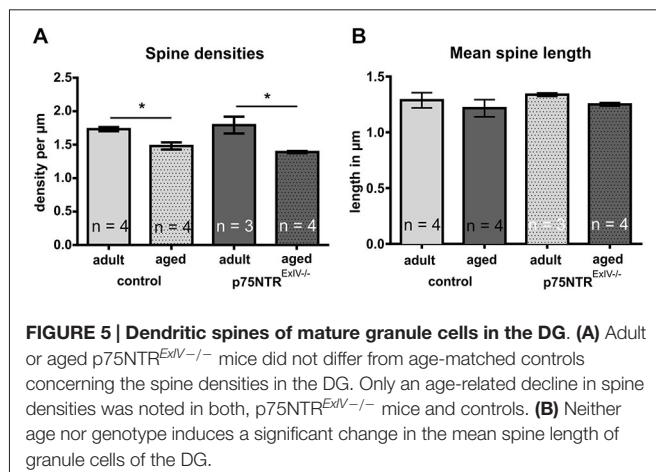


FIGURE 5 | Dendritic spines of mature granule cells in the DG. (A) Adult or aged p75NTR^{ExIV-/-} mice did not differ from age-matched controls concerning the spine densities in the DG. Only an age-related decline in spine densities was noted in both, p75NTR^{ExIV-/-} mice and controls. **(B)** Neither age nor genotype induces a significant change in the mean spine length of granule cells of the DG.

decline in spine densities from 17.93 to 13.71 spines per 10 μm ($p = 0.011$) was found. Concerning the mean length of dendritic spines, subtle alteration in mean spine length was noted by comparing p75NTR^{ExIV-/-} mice with age-matched controls; however, these effects were not significant (Figure 5B).

Discussion

Previous studies have shown that deletion of the p75 neurotrophin receptor affects hippocampal morphology, adult neurogenesis and hippocampus-related behavior (Wright et al., 2004; Catts et al., 2008; Bernabeu and Longo, 2010; Colditz et al., 2010). However, most studies were done on p75NTR^{ExIII} deficient mice, which still express s-p75NTR (Nykjaer et al., 2005) and revealed contradictory results. To overcome this obstacle, we used mice with a deletion of Exon IV resulting in a loss of both, the full-length and the short isoform of p75NTR (von Schack et al., 2001). Here, we show that p75NTR^{ExIV} deficient mice display morphological alterations in the adult DG, including an increase in thickness of the molecular and the granular layer of the DG.

Given that the DG is one of the two brain structures possessing a life-long regenerative capacity, we were interested in determining the morphological substrates that might account for the observed phenotype, since the cholinergic system, among others, may positively modulate adult hippocampal neurogenesis

(Bruel-Jungerman et al., 2011). The hippocampus receives several inputs from the basal forebrain cholinergic system. It is known that deletion of p75NTR in mice leads to an increase in the number of cholinergic neurons in the septum. This has been demonstrated for p75NTR^{ExIII-/-} mice as well as for p75NTR^{ExIV-/-} mice, although the rise in cholinergic neuron numbers was found to be more pronounced in p75NTR^{ExIV-/-} mice (Naumann et al., 2002). We were able to show that the cholinergic innervation is also increased in the DG of p75NTR^{ExIV-/-} mice, which is likely to be a consequence of the increased numbers of cholinergic forebrain neurons. In contrast to p75NTR^{ExIII-/-} mice (Dokter et al., 2015), this increase in cholinergic innervation persisted throughout age in the p75NTR^{ExIV-/-} mice.

Acetylcholine is known to play an important role in learning and defects of the cholinergic systems are associated with aging and Alzheimer's disease (AD; Muir, 1997). Age-related decline of memory function is accompanied by morphological alterations in the hippocampus (von Bohlen und Halbach and Unsicker, 2002) along with enhanced expression of p75NTR (Costantini et al., 2006). Brains affected by AD suffer from a severe decline of the cholinergic system (Terry and Davies, 1980) and p75NTR is thought to be involved in the pathogenesis of AD, including formation of tangles in the process of AD, mediating amyloid- β toxicity and stimulating amyloidogenesis (Hu et al., 2002; Chakravarthy et al., 2012). Interestingly, hippocampal p75NTR levels have been found to be increased in AD (Chakravarthy et al., 2012) and previous reports indicate that polymorphism in p75NTR are associated with a decreased risk for AD (Cozza et al., 2008; Cheng et al., 2012). Based on our current data, one could speculate that reduced expression of p75NTR in aged animals protects from age-related decline of the cholinergic system. It would be of great value to further determine whether p75NTR deficiency protects from the formation of tangles in AD pathogenesis.

Selective neurotoxic lesion of forebrain cholinergic input, e.g., with 192 IgG-saporin, was reported to reduce adult neurogenesis within the DG (Mohapel et al., 2005; Fréchette et al., 2009). Thus, we followed the idea that adult neurogenesis might be altered in p75NTR^{ExIV-/-} mice, thereby contributing to the morphological alterations of the DG. However, no effect was seen on the level of cell proliferation. Adult p75NTR knockout mice had increased numbers of newly formed DCX-positive neurons. In contrast to the cholinergic innervation, we were not able to detect a substantial increase in the numbers of DCX positive cells in aged p75NTR^{ExIV-/-} mice. Since adult p75NTR^{ExIV-/-} mice showed significant signs for elevated levels of neurogenesis, we next analyzed the population of DCX positive cells in more detail. Our results indicate that deletion of both forms of p75NTR increases the population of young postmitotic DCX-positive neurons. Moreover, p75NTR^{ExIV} deficient mice not only have elevated numbers of newborn neurons, but also more complex dendritic trees within that cell population, indicating altered neuronal differentiation.

P75NTR was shown to be expressed in the DG (Barrett et al., 2005) and recent fate-mapping experiments revealed its expression by progenitor cells located in the subgranular

zone (SGZ) and by cells of the neuronal lineage (Bernabeu and Longo, 2010). Therefore, the effects on the population of DCX-positive cells might not be solely related to alterations of the cholinergic system, but also directly related to a cell-autonomous function of p75NTR in newborn cells. In line with this hypothesis, neurotrophins have been reported to induce apoptosis of hippocampal neurons via p75NTR signaling (Friedman, 2000; Troy et al., 2002). Additionally, p75NTR acts as a high-affinity receptor for proNGF, which has been described to have pro-apoptotic effects in the hippocampus (Guo et al., 2013). Death signaling is mediated via intracellular binding partners of the p75NTR, resulting in c-jun kinase activation and subsequent activation of p53, Bax-like proteins and caspases (reviewed in: Underwood and Coulson, 2008). Thus, one could expect that p75NTR deficient mice should display reduced rates of cell death. Nevertheless, in a study from 2008, increased rates of cell death were shown to be present in p75NTR^{ExIII} deficient mice (Catts et al., 2008). This prompted us to determine the rate of cell death in the DG of p75NTR^{ExIV/-} mice by using activated caspase-3 as a specific marker. Using this approach, we were able to demonstrate that p75NTR^{ExIV/-} mice hold reduced rates of apoptosis in the DG.

Both, reduced apoptosis and increased neurogenesis is likely to contribute to the observed thickening of the granule layer. Moreover, the increase of cholinergic fibers together with the enhancement of dendritic complexity might be partly the cause for the increase in molecular layer thickness. As mentioned earlier, increased dendritic complexity was shown to be present in hippocampal pyramidal neurons of organotypic cell cultures derived from p75NTR^{ExIV/-} mice, but not in pyramidal neurons from p75NTR^{ExIII/-} mice (Zagrebelsky et al., 2005), indicating that only deletion of both, the full-length and short isoform of p75NTR induces this phenotype at least in organotypic cell cultures. This study also indicated a marked increase in dendritic spine densities in pyramidal neurons in organotypic cell cultures derived from p75NTR^{ExIV/-} mice (Zagrebelsky et al., 2005). We therefore speculated that spine densities of granule cells might also be affected in adult p75NTR^{ExIV} knockout mice. However, deletion of both, the full-length and the short isoform of p75NTR does not affect spine densities in granule cells in the knockout mice.

It would be interesting to further characterize the cell-specific contribution of p75NTR downstream targets such as Homologue of enhancer of split 1 and 5 (Hes1/5) and neurogenin 3, which might contribute to the phenotypes observed. Activation of p75NTR was shown to up-regulate the expression of Hes1/5 and increased expression of these genes is sufficient to decrease the number of dendrites (Salama-Cohen et al., 2005). Impairments in NGF/p75NTR activation on the other hand

can lead to high levels of neurogenin 3, which was shown to stimulate dendritic outgrowth (Salama-Cohen et al., 2006). In addition, BDNF signaling, a well-established mediator of dendritic arborization seems to signal through cypin to regulate dendrite number (Kwon et al., 2011). Thus, it may be possible that in p75NTR^{ExIV/-} mice, cypin promoted microtubule assembly (Tseng and Firestein, 2011) may be altered.

Using conditional p75NTR deficient mice, in which p75NTR was conditionally deleted in postmitotic choline-acetyl-transferase (ChAT) expressing cells, a lasting increase in the number of cholinergic neurons was observed (Boskovic et al., 2014), comparable to results shown for p75NTR^{ExIII} and p75NTR^{ExIV} deficient mice (Naumann et al., 2002). Contrary as to p75NTR^{ExIII} (Yeo et al., 1997; Greferath et al., 2012; Dokter et al., 2015) and p75NTR^{ExIV} mice, cholinergic innervation of the hippocampus is not altered in the ChAT-crep75^{in/in} mice (Boskovic et al., 2014). The ChAT-crep75^{in/in} mice, in contrast to p75^{ExIII} mutant mice (Catts et al., 2008; Dokter et al., 2015) display no alterations in the Morris water maze. Thus, it is possible that the changes in performance of the p75NTR^{ExIII/-} mice are due to elimination of p75NTR from the developing and/or adult hippocampus (Boskovic et al., 2014) and subsequent effects are directly associated with p75NTR in the hippocampus, as e.g., in adult neurogenesis. In this context, it would be beneficial to analyze whether the observed morphological changes in the DG of the p75NTR^{ExIV} knockout mice translate into hippocampal dependent behavior. Most hippocampus related behavioral test require that the animals have to move or navigate properly (e.g., dark-light box, Morris water maze). However, due to hind limb ataxia p75NTR^{ExIV/-} mice are not well-suited for those behavioral tests (von Schack et al., 2001).

In summary, our data show that deletion of both, the short and the full-length isoform of p75NTR causes altered DG morphology with an overall thickening of the involved cell layers.

Consequently, alterations in morphogenic substrates such as the quantity of cholinergic innervation as well as the amount of active neurogenesis and programmed cell death were evident. Thus, p75NTR is likely to play a role in regulating the cholinergic system and modulating adult neurogenesis by balancing neuronal differentiation and apoptotic cell death within the DG.

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