P75NTR deficiency leads to alterations of the hippocampal cholinergic innervation in adult and aged mice

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Introduction

Aging leads to specific learning impairments and behavioural deficits which are related to the hippocampus. Concerning aging, it is known that there is no substantial loss of neurons, but age-related alterations in the morphology and functions of neurons have been described. The cholinergic system is sensitive to age-related changes and herefore, we investigated whether aging affects the cholinergic innervation of the hippocampal formation by comparing adult (7 month of age) and aged (>20 month of age) mice. To analyse changes in the cholinergic innervation, we determined hippocampal cholinergic fiber density (CFD) using ChAT (Choline Acetyl Transferase) stained sections.

In the context of pathological aging, such as M. Alzheimer, a demise of the cholinergic system over time, accompanied by severe cell loss, has been observed. As the p75 neurotrophin receptor has turned out to be crucial for hippocampal cell differentiation and synaptogenesis and is thought to play a role in the maintenance of cholinergic neurons, we investigated its impact on the cholinergic system using two different lines of p75 deficient mice: p75ExIII mice, lacking the full-length receptor and p75ExIV mice, lacking both, the truncated (shortform-p75NTR) and full-length receptor. Using these mice, we also performed anti-ChAT stainings in order to estimate the CFD. Since it is unknown whether p75NTR-dependend alteration of the cholinergic system could persist during aging, we examined the CFD of aged (>20 months) p75 deficient mice of both knockout variants.

Since p75 mainly localizes to dendrites and spines within the hippocampal formation and since the cholinergic system has been shown to affect hippocampal learning and memory, we further analyzed the impact of the deletion of p75 receptors on hippocampal dendritic spines during aging.

Materials and methods

Cholinergic fiber density: Immunohistochemical stainings on Choline Acetyl Transferase (ChAT) were produced using polyclonal goat anti-ChAT IgG. Additionally, nuclei were highlighted by DAPI counterstaining. With 20x magnification, images were taken of the molecular and subgranular layer of the dentate gyrus, respectively. Upon these images a standardized grid with a total number of 400 intersection points was positioned. In order to estimate the cholinergic fiber density (CFD) the number of intersections crossed by cholinergic fibers (cho) was counted whereby the following density formula was devised:

$$\text{cholinerge fiber density } (\mathbf{CFD}) = \frac{\text{crossed intersections (cho)}}{\text{total number of intersections (400)}}$$

Dendritic spine density: Golgi-impregnations were performed. Golgi-stained material was analyzed using a 100x oil-objective. In order to estimate spine density, dendrites were reconstructed online using NeuroLucida. Dendrites from three distinct regions were designated for analysis: granule cell dendrites of the dentate gyrus, apical and basal dendrites of the pyramid cells in the CA1-region of the hippocampus. At least 20 dendrites per region were reconstructed.

Conclusion and outlook

We confirmed earlier studies, postulating no age-related decrease of cholinergic fibers within the hippocampus of wild type mice.
Based on our data, we conclude that the p75 neurotrophin receptor induces an inhibitory effect on the cholinergic innervation of the hippocampus since its complete absence (p75ExIV) leads to an increase of CFD which persists during aging. Simultaneous presence of the short form p75NTR (p75ExIII) provides residual functionality insofar as it leads to a milder procholinergic effect in adult mice and even a normalization of CFD in aged mice. However, despite their increased CFD, aged p75ExIV knockout mice did not show a correlating gain of dendritic spine density. Thus, it remains doubtful whether increased CFD results in stronger synaptic transmission. Accordingly, the latter issue will be scrutinized in our prospective studies.

Results

$p75^{ExIII}$

$p75^{ExIII}$ is a knockout strain descending from C57BL/6 mice. Deactivation of exons 2, 3 and 4 disables the low-affinity binding site of p75. Thus, only a shorter form of the receptor remains (s-p75NTR), whose functionality is still unresolved.

$p75^{ExIV}$

When it became clear that the receptors of $p75^{ExIII}$ mice were only partially disabled, p75ExIV was developed by additional deletion of exon 1 to represent a complete knockout of the p75NTR.

Densities of ChAT-positive fibers - *Comparison of adult and aged control mice*

Fig. 1: Comparison of adult and aged control mice showed no significant alterations of CFD. These findings approve that there is no substantial loss of cholinergic fibers within the hippocampus during aging.

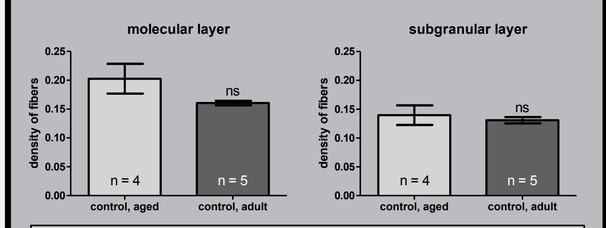


Fig. 1a - CFD of aged and adult wildtype mice (original stem of $p75^{ExIII}$):
No significant changes were measurable.

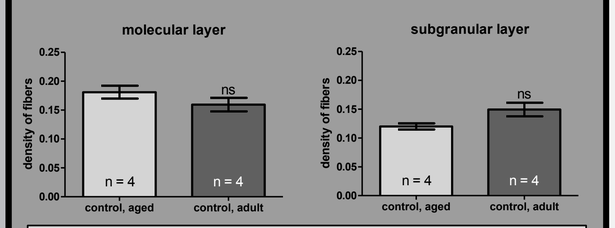


Fig. 1b - CFD of aged and adult wildtype mice (original stem of $p75^{ExIV}$):
We determined no significant alterations.

Densities of ChAT-positive fibers - *Comparison of adult knockout and control mice*

Fig. 2: In adult mice, p75NTR deficiency causes a significant increase in CFD. In presence of the truncated receptor (in $p75^{ExIII}$), the alteration of CFD is less marked.

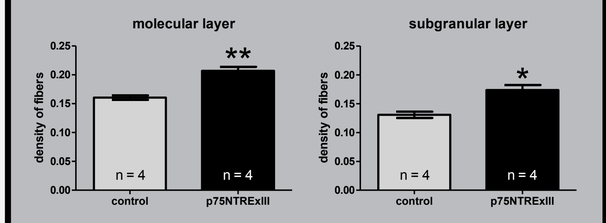


Fig. 2a - CFD of adult (7 months of age) wildtype and $p75^{ExIII}$ knockout mice:
Lack of the full length p75 neurotrophin receptor leads to a significant increase of CFD in the molecular and the subgranular layer.

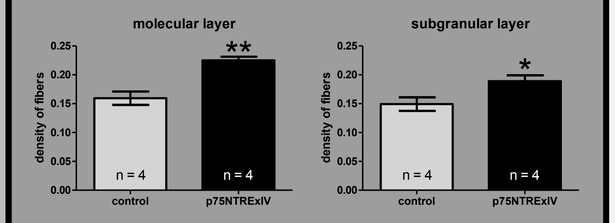


Fig. 2b - CFD of adult (7 months of age) wildtype and $p75^{ExIV}$ knockout mice:
The complete loss of the p75 neurotrophin receptor leads to a significant increase of CFD in the molecular and the subgranular layer.

Densities of ChAT-positive fibers - *Comparison of aged knockout and control mice*

Fig. 3: Only the complete knockout (p75ExIV) maintained the increased CFD during aging. Aged mice still containing the s-p75NTR (ExIII) showed no significant change in CFD compared to their aged wild type littermates.

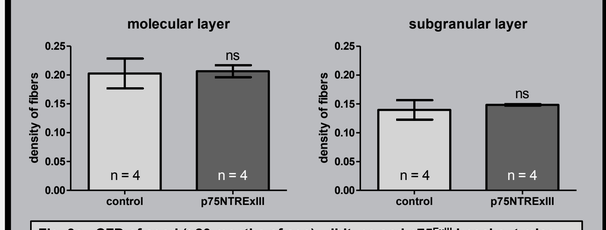


Fig. 3a - CFD of aged (>20 months of age) wildtype and $p75^{ExIII}$ knockout mice:
No significant changes were found. Thus, in the presence of s-p75NTR, alterations of the CFD appear to normalize during aging.

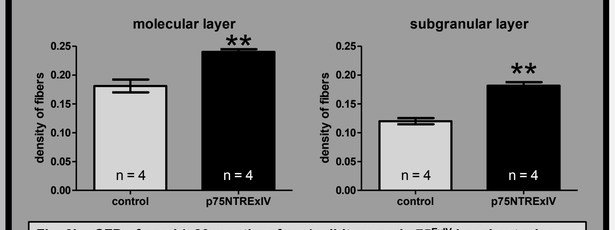
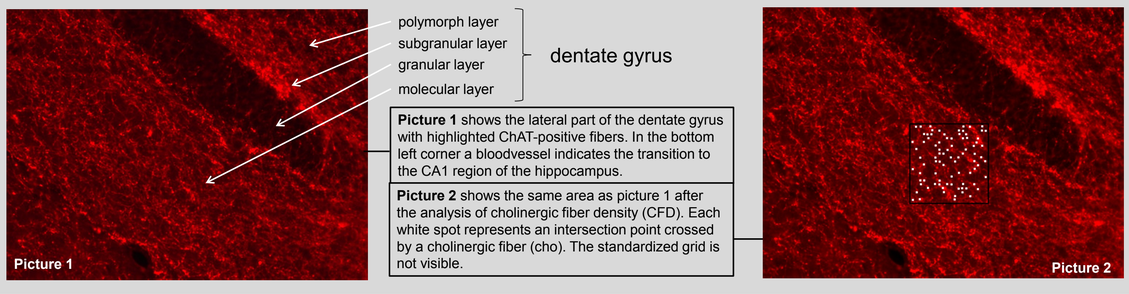


Fig. 3b - CFD of aged (>20 months of age) wildtype and $p75^{ExIV}$ knockout mice:
In mice with a complete deletion of p75NTR procholinergic effects are preserved during aging. Similar to *Figure 2b*, a significant increase in CFD was observed.



Picture 1 shows the lateral part of the dentate gyrus with highlighted ChAT-positive fibers. In the bottom left corner a bloodvessel indicates the transition to the CA1 region of the hippocampus.

Picture 2 shows the same area as picture 1 after the analysis of cholinergic fiber density (CFD). Each white spot represents an intersection point crossed by a cholinergic fiber (cho). The standardized grid is not visible.

Dendritic spine densities - *Comparison of aged knockout and control mice*

Fig. 4: Previous studies had revealed, that p75NTR deficiency leads to a gain of dendritic spine density in adult mice. In contrast, our current study suggests that spine densities of aged p75 deficient mice are not increased (possibly even decreased, see Fig. 4b).

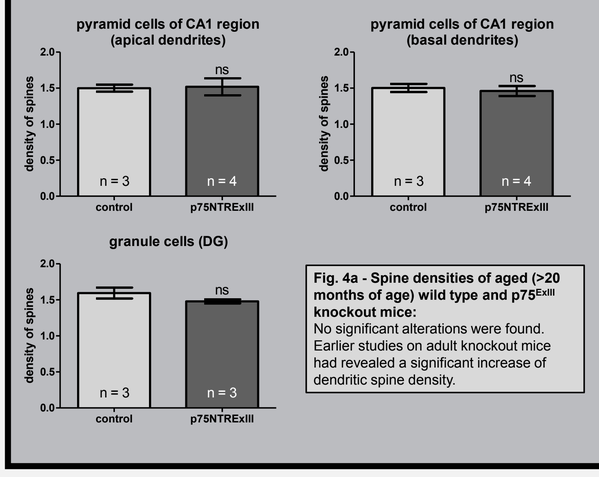


Fig. 4a - Spine densities of aged (>20 months of age) wild type and $p75^{ExIII}$ knockout mice:
No significant alterations were found. Earlier studies on adult knockout mice had revealed a significant increase of dendritic spine density.

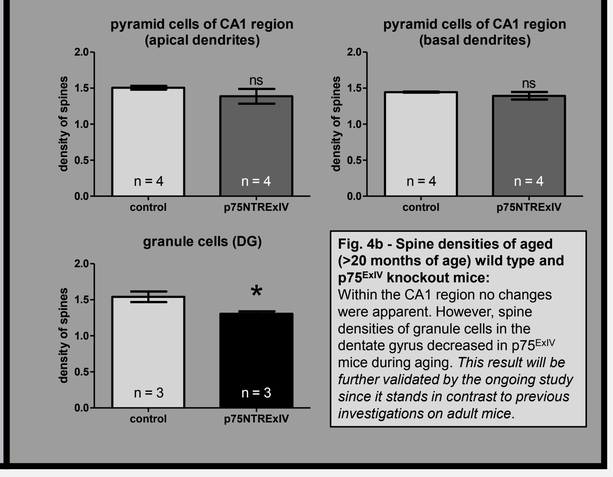


Fig. 4b - Spine densities of aged (>20 months of age) wild type and $p75^{ExIV}$ knockout mice:
Within the CA1 region no changes were apparent. However, spine densities of granule cells in the dentate gyrus decreased in $p75^{ExIV}$ mice during aging. *This result will be further validated by the ongoing study since it stands in contrast to previous investigations on adult mice.*

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