Age-related Changes in Cholinergic Activity in the CNS

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INTRODUCTION (Click)

The hippocampus is an important structure for learning and the formation of memories. This system is reliant on the release of acetylcholine, which is largely produced within the forebrain septum and then utilized by the hippocampus (Blake et al, 2014; Mcquiston, 2014; Deiana et al, 2011; Easton et al, 2012; Power et al, 2003). This relationship between the hippocampus and the septum is important for the function of the hippocampus and is thought to play a major role in cognitive loss related with aging (Feng et al., 2015). The results of prior research, mainly with neural degenerative models, has suggested that the deceased cognitive function that occurs in aging may be associated with hypofunction in cholinergic activity in the hippocampus and basal forebrain and possibly associated with a deficit in neurotrophic factors, particularly NGF (Chen and Mobley, 2019; Bartus, 1982). The goal of this study was to determine if, in normally aged mice, decreases in cholinergic activity and NGF expression are detected in the basal forebrain and hippocampal cholinergic complex.

The specific aims of this research were 1) to assess cholinergic neuronal numbers and activity through examination of choline acetyltransferase (ChAT) expression and distribution and acetylcholinesterase (ACHE) activity within the medial septal and hippocampus with young and aged mice to determine if cholinergic hypofunction is associated with normal aging; 2) assess alterations in myelin and axonal integrity within hippocampal regions through the examination of myelin basic protein (MBP) and neurofilament (NFM) expression and distribution to determine if normal aging is a neural degenerative process; 3) assess the expression of NGF within the hippocampus and medial septum to determine if a decrease in NGF accompanies normal aging.

MATERIALS and METHODS (Click)

Mouse brains were harvested from young (9-12 months, n=5) and aged (19-24 months, n=6) BalBc mice, snap-frozen in isopentane cooled in liquid nitrogen, and stored at 80°C until cryosectioned. 14µm cryosections were mounted consecutively on a series of ten slides to allow comparison of Karnovsky–Root staining for AChE activity and immunostaining.

Cryosections were immunostained using standard protocols for myelin basic protein (MBP) and neurofilament (NFM), markers for myelin and axonal integrity, NGF, and with ChAT, a cholinergic neuron marker. Digital images of hippocampus and septal regions were acquired under epifluorescence with a Zeiss MRm digital camera and Axiovision software and then assembled into collages for analysis using Adobe Photoshop. To assess AChE activity, cryosections were stained according to a modified Karnovsky–Root protocol. Digital images of the whole hippocampus and medial septal areas were obtained with a Leica Aperio Versa system. The collages from immunostained sections and digital images of Karnovsky root-stained sections were histogram–matched and thresholded to produce a binary image to eliminate bias and analyzed using Image Pro Plus software. The percentage of the total selected area stained was calculated for 3 representative sections through the hippocampus and septum. Statistical differences in the distribution of AChE and stained immunomarkers were assessed using Statistica (StatsSoft) with significance at p<0.05 by ANOVA, LSD test.

RESULTS (Click)

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In conclusion, the results of this study suggest that profound demyelination or a decrease in cholinergic neurons are not features of normal aging in the hippocampus or septum. However, normal aging my include alterations in cholinergic function and trophic support of cholinergic neurons within the septum.

CONCLUSION (Click)

The percentage of the total selected area stained was calculated for 3 thresholded to produce a binary image to eliminate bias and analyzed using Image Pro software. The percentage of the total selected area stained was calculated for 3 representative sections through the hippocampus and septum. Statistical differences in the distribution of AChE and stained immunomarkers were assessed using Statistica (StatsSoft) with significance at p<0.05 by ANOVA, LSD test.

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*All authors participated equally.
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RESULTS

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* p<0.05, ANOVA and post-hoc LSD

**AChE in the Septal Region**

Representative collages of a cryosections of the hippocampus from young and aged mouse brain immunostained for ChAT (red) and NGF (green). No significant difference was detected in immunostaining for ChAT between young and aged brain (left panel, mean + STDEV), indicating that the number of cholinergic neurons within the hippocampus did not alter with age (left panel, mean + STDEV). Immunostaining for NGF did differ significantly in total density between young and aged brain (right panel, mean + STDEV), suggesting an increase in the maintenance of this trophic factor with normal aging in the hippocampus.

* p>0.05 by ANOVA and LSD post-hoc test

**ChAT/NGF- Hippocampus**

Representative collages of a cryosections of the hippocampus from young and aged mouse brain immunostained for MBP, a marker for neurodegeneration (red) and NFM, a marker for neuronal processes (green). No significant difference in immunostaining for MBP was detected between young and aged (left panel, mean+ STDEV), indicating that significant demyelination did not accompany aging. However, the relative density of NFM immunostaining was significantly greater in the young mouse brain (right chart panel, mean+ STDEV), suggesting a loss of neurons or neuronal processes.

* p<0.05 , ANOVA and post-hoc LSD

**ChAT/NGF- Septum**

ChAT and NGF immunostaining are shown to detect differences between young and aged mouse brain in the septal region. No significant differences were detected in ChAT (left panel, mean + STDEV) or NGF (right panel, mean + STDEV), indicating that the number of cholinergic neurons did not change with age. However, ChAT and NGF immunostaining did differ significantly in total density between young and aged brain (right panel, mean + STDEV), suggesting an increase in the maintenance of this trophic factor with normal aging in the hippocampus.

* p>0.05 by ANOVA and LSD post-hoc test

**MBP and NFM**

% Area Covered

Representative collages of a cryosections of the forebrain septal region from young and aged mouse brain immunostained for MBP, a marker for neurodegeneration (red) and NFM, a marker for neuronal processes (green). No significant difference in immunostaining for MBP was detected between young and aged (left panel, mean+ STDEV), indicating that significant demyelination did not accompany aging. However, the relative density of NFM immunostaining was significantly greater in the young mouse brain (right chart panel, mean+ STDEV), suggesting a loss of neurons or neuronal processes.

* p<0.05 , ANOVA and post-hoc LSD

**ChAT/NGF**

% Area Covered

Representative collages of a cryosections of the forebrain septal region from young and aged mouse brain immunostained for ChAT (red) and NGF (green). No significant difference was detected in immunostaining for ChAT between young and aged brain, indicating that the number of cholinergic neurons within the septum did not alter with age (left panel, mean + STDEV). However, immunostaining for NGF did differ significantly in total density between young and aged brain with aged septum expressing increased NGF (right panel, mean + STDEV), suggesting an increase in the maintenance of this trophic factor with normal aging in the hippocampus.

* p>0.05 by ANOVA and LSD post-hoc test

**Representative collages of a cryosections of the hippocampus from young and aged mouse brain stained by Karnovsky-Roots technique for AChE. Significantly increased activity was detected in the hippocampus of young mice compared to aged (upper chart, mean + STDEV; in contrast, significantly greater activity was detected in the forebrain septum of aged mice (lower chart, mean + STDEV).**

* p>0.05 by ANOVA and LSD post-hoc test
DISCUSSION AND CONCLUSION

• No differences in ChAT expression were detected between young and aged mice, which would indicate no change in cholinergic cell number in normal aging. However, a significant decrease in AChE activity in the septum was observed concomitant with an increased NGF expression in aged mice compared to young. Previous studies suggest that NGF is produced in the hippocampus and travels to the septal region. Septal NGF levels could be increased in response to decreased plasticity of aged cholinergic neurons in the septum to provide increased trophic support and maintain normal cholinergic activity in this region. Another explanation for an increased expression of NGF in the septum could be the increased expression of the pro-NGF form of the growth factor. Antibodies used in this study were non-selective to the pro-NGF form versus the mature NGF form. The pro-NGF form of the growth factor binds preferentially to the p75NTR receptor and disrupts normal neurotrophic activity, resulting in apoptosis. Such a switch to a pro-NGF form has been demonstrated in neural degenerative animal models (Iulita et al, 2017) and might explain the decreased cholinergic neuronal activity in the septal region demonstrated by the decreased AChE activity.

• There was an observed increase in AChE activity and a decrease in the amount of NFM within the older mouse hippocampus. A decrease in the NFM may accompany an impaired transport of acetylcholine within the hippocampal region and the possible accumulation of unutilized acetylcholine. The increased activity of AChE within the aged hippocampus detected in this study may be a response to a need to break down the unused acetylcholine.

• Previous studies have shown that MBP expression is increased in demyelinated areas of the brain. Results from this study showed no significant difference in hippocampal MBP expression between old and young mice. This could indicate that normal aging is not associated with rapid demyelination in this region.

In conclusion, the results of this study suggest that profound demyelination and a decrease in cholinergic neurons are not features of normal aging in the hippocampus or septum. However, normal aging may include alterations in cholinergic function and trophic support of cholinergic neurons within the septum.

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