

MMP-3 deficiency does not influence the length and number of CA1 dendrites of hippocampus of adult mice

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Over the past two decades, metalloproteinases (MMPs), including MMP-2, MMP-3, and MMP-9, have been implicated as important players in mechanisms underlying various forms of neuroplasticity. In particular, MMP-3 was found to be involved in both cognitive functions and in plasticity phenomena, but the underlying molecular mechanisms remain largely elusive. In general, it is believed that functional plasticity of neurons is associated with morphological alterations. Interestingly, MMP-9, in addition to playing a key role in synaptic plasticity, was found to affect plasticity-related spine morphology changes. Whereas the involvement of MMP-3 in shaping synapse morphology upon induction of synaptic plasticity awaits determination, it has been demonstrated that MMP-3 knockout results in clearly altered apical dendrite morphology in pyramidal neurons in mouse visual cortex. Considering that the involvement of MMP-3 in synaptic plasticity has been most extensively documented for the CA1 hippocampal region, we decided to investigate whether genetic deletion of MMP-3 affects neuronal morphology in this area. To this end, we used Golgi staining to compare dendritic morphology of pyramidal neurons in the CA1 region in MMP-3-deficient and wild-type mice. Surprisingly, in contrast to the results obtained in cortex, extensive analysis of dendritic morphology in the CA1 region revealed no significant differences between MMP-3 knockout and wild-type groups. These results suggest that the impact of MMP-3 on neuronal morphology may be region-specific.

Key words: matrix metalloproteinase, Golgi staining, hippocampus, CA1, neuron, dendrite, microscopy

INTRODUCTION

Matrix metalloproteinases (MMPs) are present in many types of tissues, playing a variety of physiological and pathological roles (Ethell and Ethell 2007, Rivera et al. 2010). Over approximately the past two decades, these enzymes were found to be prominently expressed in the brain, especially MMP-2, MMP-3 and MMP-9, and to play an important role in various neuroplasticity phenomena in the central nervous system (CNS). In particular, MMP-mediated proteolysis of extracellular matrix components, other proteases or cell adhesion molecules have been implicated as important components of signaling pathways underlying various

types of neuroplasticity (Brzdak et al. 2017a, Ethell and Ethell 2007, Lee et al. 2008, Nagy et al. 2006, Tsilibary et al. 2014, Vafadari et al. 2016). Moreover, several studies have suggested that these enzymes participate in a variety of functions in the developing, as well as adult, CNS, particularly in synaptogenesis, synaptic plasticity, synapse formation or remodeling (Jaworski 2000, Sekine-Aizawa et al. 2001).

Among the metalloendopeptidases, MMP-3 emerges as an important player in cognitive function (Meighan et al. 2006, Olson et al. 2008, Wright et al. 2006) and plasticity phenomena (Van Hove et al. 2012a), yet the molecular mechanisms by which MMP-3 impacts these processes are only starting to be revealed. It has been shown, for instance, that inhibition of MMP-3 activity

or a deficit of MMP-3 protein causes deficiency in associative memory formation (Wright et al. 2006), impairments in maintaining hippocampal long term potentiation (LTP) (Wiera et al. 2017) or weakness of motor functions such as coordination, learning and balance (Van Hove et al. 2012b). MMP-3 has also been implicated in the pathogenesis of various brain disorders, in particular Alzheimer's disease, Parkinson's disease and multiple sclerosis (Brzdak et al. 2017a, Van Hove et al. 2012a).

It is widely accepted that functional plasticity of neurons is associated with morphological alterations. Most notably, LTP induction is accompanied by remodeling of dendritic spines towards a mushroom-like morphology (Muller et al. 2000, Yuste and Bonhoeffer 2001). Interestingly, MMP-9, in addition to playing a key role in synaptic plasticity, was found to affect plasticity-related spine morphology changes (Michaluk et al. 2011, Szepesi et al. 2013, Wang et al. 2008). These findings indicate a key role for MMP-9 in both functional and structural manifestations of synaptic plasticity. Thus, it is interesting to address the question of whether MMP-3 is involved in regulating neuronal morphology. To our knowledge, the only report addressing this issue is Aerts et al. (2015), in which the authors demonstrated marked impairments in cross-modal plasticity in the visual cortex after monocular enucleation in MMP-3-deficient mice. Moreover, apical dendrites of pyramidal neurons in layer V of the visual cortex of MMP-3 KO mice showed shorter length and more abundant branching than those in the wild-type (WT) animals. Considering that involvement of MMP-3 in synaptic plasticity has been most extensively documented for the CA1 hippocampal region, we decided to investigate whether genetic deletion of MMP-3 affects the neuronal morphology in this hippocampal area. To this end, we used Golgi staining (Chan-Palay and Palay 1972, Fairen et al. 1977, Zaquout and Kaindl 2016) to compare dendritic morphology of pyramidal neurons in the CA1 region in MMP-3-deficient and WT mice. Surprisingly, in contrast to results obtained in the cortex by Aerts et al. (2015), extensive analysis of dendritic morphology in the CA1 region revealed no significant differences between the MMP-3 KO and WT groups. These results suggest that the impact of MMP-3 on neuronal morphology may be region-specific.

METHODS

Animals

Procedures were performed in adult (3 months old) female C57BL/6 mice, two wild-type (WT) and

two MMP-3 deficient (MMP-3^{-/-}) mice (Neural Circuit Development and Regeneration Research Group, University of Leuven, Belgium), confirmed by genotyping. Mice were kept under standard laboratory conditions under a 14 h/10 h dark/light cycle with food and water *ad libitum*. All the animal experimental procedures were in accordance with the Polish and Belgian Animal Welfare Acts.

Golgi staining

WT and MMP-3^{-/-} mice were anesthetized with isoflurane inhalation and decapitated. Brains were dissected, quickly rinsed with deionized H₂O, and immersed in the Golgi staining solution (FD Rapid GolgiStain™ Kit, FD NeuroTechnologies Inc., MD, USA) for 2 weeks at room temperature in the dark. The solution was exchanged 6 hours after the first immersion. Then tissue was transferred into the protection solution, which was replaced after the first 24 hours, and stored for 3 days at room temperature in the dark. Brains were sectioned into 100 µm thick slices using a vibratome (Leica VT1000S), and mounted on glass microscope slides (Superfrost Plus, Thermo Scientific). The air-dried sections were stained according to the manufacturer's protocol, and stored in darkness before imaging.

Image acquisition and analysis

Bright field microscopy images were acquired using an Olympus BX41 microscope using Cell D software. The Z-stack images were analyzed using ImageJ software (Rueden et al. 2017). Hippocampal CA1 dendrites were semi-automatically traced with the NeuronJ plugin available for the ImageJ software. Only pyramidal neurons with proper impregnation of the Golgi staining, that were minimally obscured by other cells and had an unimpaired dendritic tree, were selected for analysis. For each genotype (WT and MMP-3^{-/-}) 30 cells were analyzed. All obtained tracings were checked against the original Z-stack data to exclude possible mistakes. For each reconstructed neuron, the length of the basal dendrites, the apical shaft length, the number of dendrites and soma area were determined.

Statistical analysis

Statistical analysis was performed using SigmaPlot, and $\alpha=0.05$ was chosen for statistical significance. In a single comparison, significance was calculated for data with a normal distribution using Student's *t* test,

and for data that did not pass the normality test, the Mann-Whitney U test was used. The tests that were used in specific experiments are disclosed in the figure legends. In all comparisons, data is presented as the mean $\pm$ standard error of the mean (SEM) and *n* refers to the number of examined cells.

RESULTS

Considering that genetic deletion of MMP-3 resulted in pronounced changes in neuronal morphology in the visual cortex (Aerts et al. 2015), we used Golgi staining to analyze the impact of MMP-3 knockout on pyramidal neurons in the CA1 region of the hippocampus. As shown in Fig. 1 A, B, this method allows for contiguous staining of the whole neuron, including soma and dendritic trees, thus enabling a detailed analysis of the morphological features of individual neurons. For this purpose, stained neurons were graphically reconstructed as shown in Fig. 1 C, D. At first glance, no

obvious differences between WT and MMP-3^{-/-} groups were apparent. Considering that Aerts et al. (2015) reported that apical dendrites from neurons in MMP-3 KO animals showed a shorter length than those in the WT group, we performed a detailed characterization of neuronal morphology, and assessed the length of the apical dendrites, the sum of the lengths of the dendrites for the same cell, and the maximum and minimum lengths (Fig. 2A-D). We compared these parameters for both groups and no significant differences were found (Fig. 2). Following analysis of the apical dendrite morphology, we investigated the basal dendritic tree characteristics. In this case, we also found that the mean length, as well as the sum of lengths, and minimum/maximum lengths did not significantly differ between WT and MMP-3^{-/-} mice (Fig. 2E-H). Since Aerts et al. (2015) reported differences in branching of dendritic trees in WT and MMP-3 groups we performed an analysis of the number of apical (Fig. 2I) and basal (Fig. 2J) dendrites, but no significant differences were found. Finally, we analyzed the size of the neuronal soma. A mi-

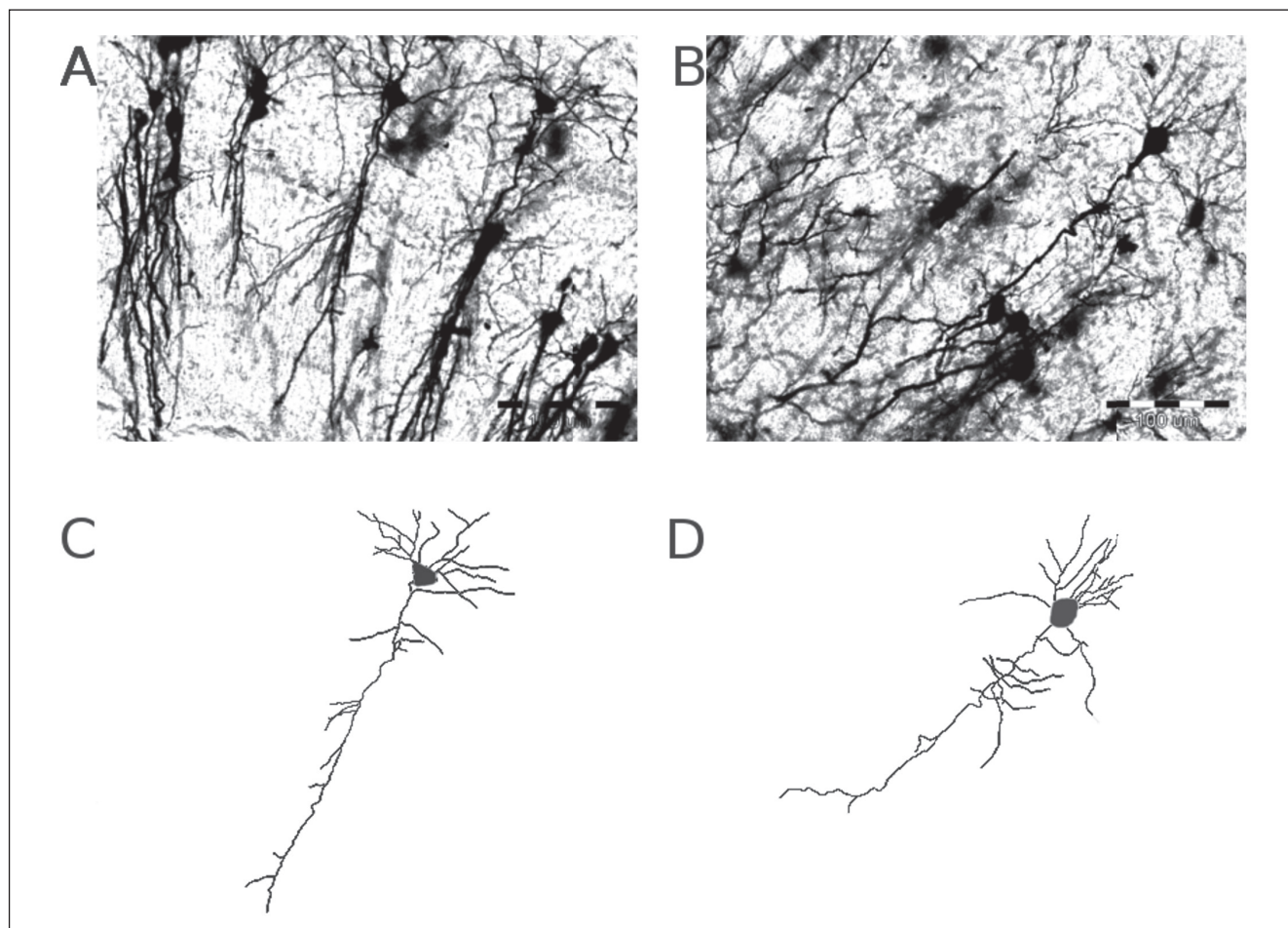


Fig. 1. General morphology of CA1 pyramidal neurons in WT and MMP-3^{-/-} mice. Representative bright field images of Golgi-stained hippocampal CA1 neurons of WT (A) and MMP-3^{-/-} (B) mice.

nor trend of soma area reduction in the MMP-3 KO was apparent, but this observation did not reach statistical significance (Fig. 2K). Altogether, in the hippocampal CA1 region, genetic deletion of MMP-3 did not result in any significant morphological alterations to the pyramidal neurons, in contrast to what has been observed in the visual cortex (Aerts et al. 2015).

DISCUSSION

The present study was inspired by the observation that genetic deletion of MMP-3 is associated with marked alterations of dendritic morphology in the pyramidal cells of the visual cortex (Aerts et al. 2015). This led us to investigate the impact of MMP-3

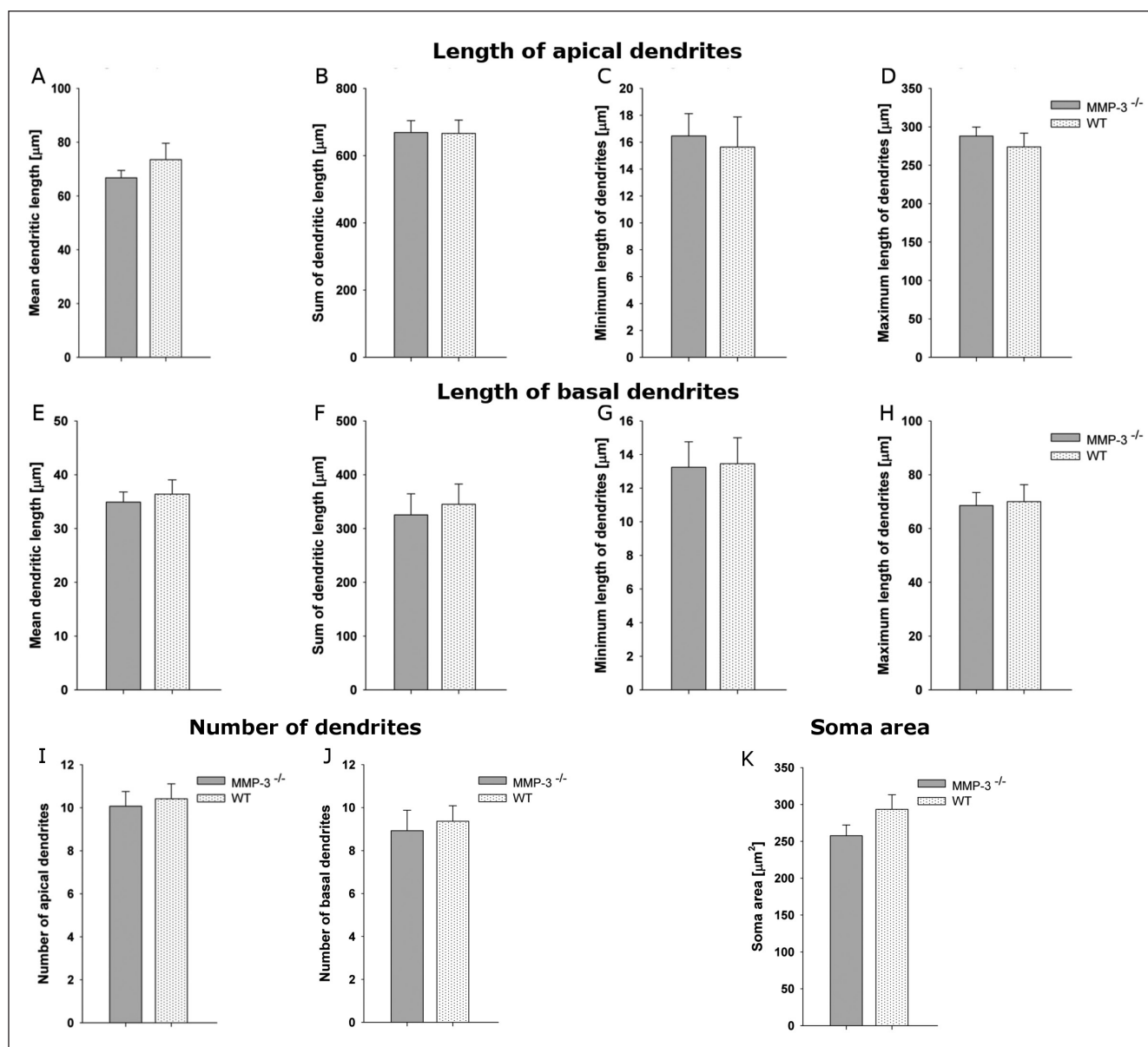


Fig. 2. Morphological evaluation of the dendritic tree of mouse hippocampal CA1 neurons. Morphological analysis of the apical dendrites [(A) MMP-3^{-/-} 66.8 ± 2.7, *n*=28; WT 73.5 ± 6.0, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.821; (B) MMP-3^{-/-} 668.7 ± 35.4, *n*=30; WT 666.2 ± 39.2, *n*=28; Student's *t* test, *P*=0.962; (C) MMP-3^{-/-} 16.5 ± 1.7, *n*=30; WT 15.6 ± 2.2, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.333; (D) MMP-3^{-/-} 288.0 ± 11.7, *n*=30; WT 273.7 ± 18.0, *n*=29; Student's *t* test, *P*=0.506] and basal dendrites [(E) MMP-3^{-/-} 34.9 ± 1.9, *n*=26; WT 36.4 ± 2.7, *n*=30; Student's *t* test, *P*=0.660; (F) MMP-3^{-/-} 325.2 ± 39.3, *n*=26; WT 345.0 ± 37.7, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.889; (G) MMP-3^{-/-} 13.3 ± 1.5, *n*=26; WT 13.5 ± 1.5, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.993; (H) MMP-3^{-/-} 68.5 ± 4.9, *n*=26; WT 70.0 ± 6.3, *n*=30; Student's *t* test, *P*=0.856]. Examination of the number of dendrites in CA1 projection neurons of the hippocampus: apical dendrites [(I) MMP-3^{-/-} 10.1 ± 0.7, *n*=30; WT 10.4 ± 0.7, *n*=29; Student's *t* test, *P*=0.723], basal dendrites [(J) MMP-3^{-/-} 8.9 ± 1.0, *n*=26; WT 9.4 ± 0.7, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.520] and soma size [(K) MMP-3^{-/-} 257.6 ± 14.5, *n*=29; WT 293.3 ± 19.9, *n*=30; Mann-Whitney Rank Sum Test, *P*=0.175]. Bars indicate means ± SEM.

deficiency on the morphology of pyramidal neurons in the CA1 hippocampal region, where plasticity phenomena and their dependence on MMP-3 has been studied in detail (Brzdak et al. 2017b, Van Hove et al. 2012b, Wiera et al. 2017). Considering these premises, it is somewhat surprising that MMP-3 deficiency has no effect on dendritic morphology in this region of the hippocampus. Although the exact reason for these diverging phenotypes in the cortex versus CA1 hippocampal region is not known, studies into the mechanisms of neuroplasticity have previously revealed a myriad of molecular mechanisms that may differ in different brain regions. Interestingly, we have demonstrated in our recent study (Wiera et al. 2017) that NMDA receptor- and voltage-gated calcium channel-dependent hippocampal LTP are associated with activity of different MMPs, i.e., of MMP-9 and MMP-3, respectively. This points to the important notion that distinct types of plasticity phenomena may rely on different MMPs. Indeed, mechanisms underlying structural alterations of the synapse, most commonly the dendritic spine, which typically accompany synaptic plasticity, are expected to rely on distinct molecular pathways relative to those underlying more global changes in dendritic architecture, occurring over a longer time scale. Moreover, both synaptic plasticity and global dendritic structural plasticity are known to be stimulated by behavioral performance associated with cognition (see Tavosanis 2012, for review), but the latter occurs at a much longer time scale (e.g. upon enhanced cognition in enriched environment or upon prolonged stress), giving rise to more extensive alterations in the dendritic architecture and thus relying on more complex mechanisms. Our data interestingly demonstrate that involvement of MMP-3 in controlling long-term changes in dendritic morphology may be region-specific, and that in hippocampal pyramidal neurons the latter process does not rely on MMP-3, whereas in the cortex it does. It remains to be established to what extent the involvement of MMP-3 in synaptic plasticity in hippocampal and cortical neurons affects synaptic morphology. The observed difference in phenotype may be a result of MMP-3 deficiency differentially effecting cortical versus hippocampal development. The exact timing of neuronal development and mechanisms of development can differ and/or differently rely on the presence of MMP-3. In conclusion, we have provided the first analysis of the role of MMP-3 in shaping the dendritic architecture in the hippocampal CA1 region, showing that, in contrast to cortical neurons, the deficit of this metalloproteinase has no effect on the morphologic features of these neurons, thus suggesting a region-specific role for MMP-3.

CONCLUSIONS

In the present study we investigated whether genetic deletion of MMP-3 affects the length and branching of pyramidal neurons in the CA1 hippocampal region, where this MMP was shown to affect synaptic plasticity. Extensive morphological analysis of Golgi-stained neurons revealed no significant differences in apical and basal dendrites or in soma size between MMP-3 knockout and WT groups. These findings differ from previously published results for pyramidal neurons in the visual cortex, indicating that the involvement of MMP-3 in regulating neuronal morphology may be region-specific.

ACKNOWLEDGMENTS

The authors were supported by National Science Center grant OPUS/2014/15/B/NZ4/01689. We thank Prof. Dziegiel (Department of Human Morphology and Embryology Division of Histology and Embryology) for access to the bright microscope (Olympus BX41) used for image acquisition.

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