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Author manuscript

*Neurobiol Dis.* Author manuscript; available in PMC 2021 February 03.

Published in final edited form as:

*Neurobiol Dis.* 2021 January ; 148: 105150. doi:10.1016/j.nbd.2020.105150.

## A new role for matrix metalloproteinase-3 in the NGF metabolic pathway: proteolysis of mature NGF and sex-specific differences in the continuum of Alzheimer's pathology

Rowan Pentz<sup>a</sup>, M. Florencia Iulita<sup>b,c</sup>, Maya Mikutra-Cencora<sup>b</sup>, Adriana Ducatenzeiler<sup>b</sup>, David A. Bennett<sup>d</sup>, A. Claudio Cuello<sup>a,b,e,f</sup>

<sup>a</sup>Department of Neurology and Neurosurgery, McGill University, Montreal, Canada

<sup>b</sup>Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada

<sup>c</sup>Sant Pau Memory Unit, Department of Neurology, Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

<sup>d</sup>Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, Illinois, USA

<sup>e</sup>Department of Anatomy and Cell Biology, McGill University, Montreal, Canada

<sup>f</sup>Department of Pharmacology, Oxford University, US (Visiting Professor)

### Abstract

Matrix metalloproteinase-3 (MMP-3) has been associated with risk of Alzheimer's disease (AD). In this study we introduce a novel role for MMP-3 in degrading nerve growth factor (NGF) *in vivo* and examine its mRNA and protein expression across the continuum of AD pathology. We provide evidence that MMP-3 participates in the degradation of mature NGF *in vitro* and *in vivo* and that it is secreted from the rat cerebral cortex in an activity-dependent manner. We show that cortical MMP-3 is upregulated in the McGill-R-Thy1-APP transgenic rat model of AD-like amyloidosis. A similar upregulation was found in AD and MCI brains as well as in cognitively normal individuals with elevated amyloid deposition. We also observed that frontal cortex MMP-3 protein levels are higher in males. MMP-3 protein correlated with more AD neuropathology, markers of NGF metabolism, and lower cognitive scores in males but not in females. These results suggest that MMP-3 upregulation in AD might contribute to NGF dysmetabolism, and therefore to

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**Corresponding author:** A. Claudio Cuello, OC, MD, DSc, FRSC, FMedSi, Department of Pharmacology and Therapeutics, Faculty of Medicine, McGill University, 3655 Sir-William-Osler Promenade, Room 1210, Montreal, QC, Canada, H3G 1Y6, Tel: 514-398-3618; Fax: 514-398-8317, claudio.cuello@mcgill.ca.

Authors' contributions

RP, MFI and ACC conceived and designed the study. RP performed all experiments save the *in vitro* degradation assays, which were performed by AD, with experimental support by MM-C. MFI, AD, and ACC provided input into experimental and analytic methods. DAB provided brain tissue as well as the corresponding neuropathological and cognitive data. RP analyzed the data and prepared the figures. The manuscript was written by RP and ACC with input from all other authors. All authors have read, revised, and approved the submitted manuscript.

Conflict of interest

The authors have no conflict of interest to report.

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cholinergic atrophy and cognitive deficits, in a sex-specific manner. MMP-3 should be further investigated as a biomarker candidate or as a therapeutic target in AD.

### Keywords

Alzheimer's disease; basal forebrain cholinergic neurons; Nerve Growth Factor; NGF Metabolic pathway; proNGF; Matrix Metalloproteinase-3

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### Introduction

Matrix metalloproteinase-3 (MMP-3) is a multifunctional brain protease with key roles in the developing and adult nervous system. Polymorphisms in the coding region of MMP-3 have been linked to increased risk of Alzheimer's disease (AD) (Helbecque et al., 2007; Saarela et al., 2004), while MMP-3 in cerebrospinal fluid (CSF) and in plasma has been demonstrated to be altered in AD and to correlate with cognitive impairment and core AD biomarkers (Hanzel et al., 2014; Horstmann et al., 2010; Iulita et al., 2019; Peng et al., 2015; Stomrud et al., 2010a). A recent longitudinal study demonstrated that MMP-3 levels are elevated early in AD plasma and confer risk of AD-related cognitive decline in females, but not males (Iulita et al., 2019).

Basal forebrain cholinergic neurons (BFCNs) depend on a target-derived supply of nerve growth factor (NGF) from cortical target cells for the maintenance of their cholinergic function and phenotype (Cuello, 1993; Garofalo et al., 1992; Hefti, 1986). Mature NGF (mNGF) is produced from its precursor (proNGF) by the plasmin activating system and is subsequently degraded by matrix metallo-proteinase-9 (MMP-9) (Bruno and Cuello, 2006). The enzymes and zymogens involved in the NGF metabolic pathway are secreted at cortical/hippocampal cholinergic terminal regions in response to glutamatergic or cholinergic stimulation to produce a controlled trophic response (Bruno and Cuello, 2006).

Beginning early in the AD pathological process, the production of mNGF is insufficient and the degradation of mNGF is excessive (Bruno et al., 2009a; Bruno et al., 2009b; Pentz et al., 2020). This interruption of trophic support explains the progressive atrophy of the BFC projection system in AD (Pearson et al., 1983; Whitehouse et al., 1982), a neurotransmitter system that is critical for the maintenance of higher CNS functions such as learning, attention, and memory (Bartus et al., 1982; Drachman and Leavitt, 1974; Everitt and Robbins, 1997; Hasselmo and Sarter, 2011; Mesulam, 2004).

We were interested in the potential role of MMP-3 in mNGF proteolysis for the following reasons: MMP-3 has been observed to be secreted in response to cholinergic stimulation in peripheral tissues (De Couto et al., 2009; Reina et al., 2011), MMP-3 consensus target sequences overlap with MMP-9 consensus target sequences (Eckhard et al., 2016), MMP-3 can activate MMP-9, the main NGF-degrading protease (Ogata et al., 1992), MMP-3 is upregulated in AD plasma and CSF (Hanzel et al., 2014), and MMP-3 has also been reported to differentially modulate AD-related cognitive decline by sex (Iulita et al., 2019). Therefore, we were interested in whether MMP-3 could play a direct or indirect role in

mNGF proteolysis and, furthermore, whether its expression might be altered sex-specifically throughout the continuum of AD.

In this report, we demonstrate for the first time that MMP-3 can contribute to mNGF degradation *in vitro* and *in vivo* and that it is released from cortical cells in response to cholinergic stimulation (or non-specific neuronal depolarization), like other NGF metabolic pathway proteins. We demonstrated increased MMP-3 levels in males throughout the AD continuum, beginning prior to cognitive decline in human prefrontal cortex. A similar MMP3 up-regulation was observed in a rat model of AD-like amyloidosis. Furthermore, we have found that human MMP-3 levels in AD consistently correlate to cognitive decline and measures of AD pathology in males, but not in females. The results of these studies indicate that MMP-3 should be considered as a novel member of the NGF metabolic pathway, which is altered in AD in a sex-specific manner.

## Methods

All procedures were approved by the Animal Care Committee of McGill University, in accordance with the guidelines of the Canadian Council on Animal Care. Efforts were made to minimize the number and suffering of the animals used. McGill-R-Thy1-APP transgenic rats express the Swedish double and Indiana genetic mutations in the human amyloid precursor protein gene, hA $\beta$ PP, leading to progressive amyloidosis and cognitive deficits (Iulita et al., 2014a; Leon et al., 2010). Animals were housed with controlled humidity and temperature under 12-hour light cycle and had access to food and water *ad libitum*.

### *In vitro* NGF degradation assay

Ten or twenty ng of 14 kDa recombinant human mNGF produced in *E. Coli* (N-245; Alomone labs, Jerusalem, Israel) was diluted in 50  $\mu$ l PBS and incubated with 0, 3.7, 7.4, or 14.8 mM of active recombinant human MMP-3 (ab96555; abcam, Cambridge, UK). Solutions were incubated overnight at 37 °C with agitation, then mixed with denaturing loading buffer 1:4 for Western blotting, the protocols for which are described later in the methods.

### *In vivo* NGF degradation assays

Anaesthesia was induced in six 8-month-old wild-type Wistar rats (3 males, 3 females) with 5% isoflurane in oxygen in an induction chamber and maintained with 2% isoflurane in oxygen through a face mask. Rats were stereotaxically injected with 2.0  $\mu$ l of 1.0  $\mu$ g/ $\mu$ L human recombinant MMP-3 (ab96555; Abcam, Cambridge, UK) in saline or with 2.0  $\mu$ l of 0.5  $\mu$ M of the MMP-3 inhibitor, UK356618 (5.9 nM IC<sub>50</sub>, ~150x selectivity vs. other MMPs; Santa Cruz, CA, USA) in DMSO, with vehicle injected contralaterally. The side receiving the vehicle was randomized for each injection. Injections were performed with a 5.0  $\mu$ l Hamilton microsyringe fitted with a 32 gauge needle (Hamilton, USA) in the dorsomedial hippocampus according to the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2006) at the following coordinates:  $-3.6$  antero-posterior,  $\pm 2.0$  mediolateral ( $\pm$  giving left/right, which was randomized to contralateral/ipsilateral), and  $-2.8$  dorsoventral from Bregma.

After 12–16 hours, rats were deeply and irreversibly anaesthetized by 100 mg/100 g equithesin administered intraperitoneally and perfused transcardially with cold perfusion buffer, following (Côté et al., 1993) for composition. Each dorsal hippocampus was macrodissected and divided into anterior, medial, and posterior segments. Each segment was then homogenized both manually and through sonication in RIPA buffer with CompleteMini protease inhibitors (Sigma-Aldrich, St. Louis, USA), then prepared for Western blotting as described below.

### Western blotting

Standard reducing loading buffer was added to samples at a ratio of 1:4. Each sample (8 µl of 3.0 µg/µl rat/human cortex samples, 12 µl *in vitro* assay samples, and 20 µl of superfusate fractions) was added to an SDS-polyacrylamide gel (10–15%) and run for 90 minutes at 120V. The protein contents of each gel were transferred to nitrocellulose membranes (GE Healthcare, Chicago, USA) at 0.3 A for 80 minutes. Membranes were blocked for one hour with a solution of 5% milk in TBST, then incubated overnight in appropriate antibody solutions: ab52915 anti-MMP-3 (Abcam, Cambridge, UK) was used to reveal MMP-3 immunoreactivity at 45 kDa and proMMP-3 at 57 kDa, ANT-005 (Alomone labs, Jerusalem, Israel) was used to reveal 27 kDa (unmodified) proNGF, H-20 anti-NGF (Santa-Cruz, Dallas, Texas) was used to reveal all NGF immunoreactivity in *in-vitro* studies, and the NGF 9795 (Abcam, Cambridge, UK) was used to reveal mature NGF immunoreactivity at 14 kDa in human tissue, following the protocol of Locke et al. (Locke et al., 2020) as adapted by (Pentz et al., 2020). In the latter case, a chloroform/methanol protein extraction was required to detect mNGF at the low abundance at which it exists in the human cortex, following recently published protocols (Pentz et al., 2020). The identity of the quantified band at 45 kDa was established by comparison to a positive control (recombinant MMP-3 as above or lung tissue). Peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, USA) were subsequently added at 1:10 000 in TBST for one hour at room temperature. The membranes were detected using Western Lightning Electrochemiluminescence Solution (PerkinElmer Inc, Waltham, USA) and exposed to Highblot chemiluminescence-detecting films (Denville, Canada). These films were developed using a KODAK X-OMAT 2000A (Eastman Kodak Company, Rochester, USA), and quantified using CLIQS software (Totallab, Newcastle, UK). The integrated optical density (IOD) value for each sample was normalized to the IOD of GAPDH (AB2303; Millipore, Billerica, USA) on the same membrane and to an internal control to ensure consistency between membranes; each sample was measured in triplicate. IODs are represented as a fold change from the average of the control group. Average GAPDH IODs did not differ between groups.

### Ex vivo superfusion-stimulation of rat cerebral cortex tissue

Superfusion experiments were done following the protocol of (Bruno and Cuello, 2006) to determine whether MMP-3 is released in response to cholinergic stimulation, like NGF metabolic pathway proteins. Three-month-old type Wistar adult rats (n = 4; 2 male, 2 female) were euthanized by rapid decapitation and brains rapidly removed and macrodissected. The cerebral cortices were diced with a McIlwain sectioning instrument and were placed on a semi-permeable 8.0 µm pore membrane (Corning Life Sciences, New

York, United States), submerged in Hanks buffer + 5% O<sub>2</sub> and 1% CO<sub>2</sub> and superfused at a rate of 0.25 mL/minute. The temperature was maintained at 37°C ± 2. After a 45-minute adaptation period, a baseline sample was taken. A 100 nM solution of the cholinergic agonist carbachol (or 0.05M KCl) was then added to the preparation and allowed to incubate (pumps off) for 1 minute. Further samples were taken at 5, 10, 15, and 30 minutes after the initial aliquot was drawn. The preparation was then allowed to re-equilibrate for 15 minutes, and the procedure was repeated. To assess the intracellular versus extracellular Ca<sup>2+</sup>-dependency of activity-dependent release of MMP-3, incubations were made in the presence or absence of 10 µM of either the cell-permeant calcium chelator BAPTA-AM or cell-impermeant BAPTA (Sigma-Aldrich, St. Louis, Missouri, USA), added at the beginning of the adaptation period.

### **Neurochemical analysis of McGill-R-Thy1-APP rat prefrontal cortex across multiple timepoints**

Prefrontal cortex tissue was acquired from the offspring of crossed heterozygous McGill-R-Thy1-APP transgenic rats: 7 APP<sup>-/-</sup> (3 male, 4 female), 6 APP<sup>+/-</sup> (3 male, 3 female), and 7 APP<sup>+/+</sup> (4 male, 3 female) at 3 months old, 7 APP<sup>-/-</sup> (4 male, 3 female), 6 APP<sup>+/-</sup> (4 male, 2 female), and 6 APP<sup>+/+</sup> (3 male, 3 female) at 6 months old, and 14 APP<sup>-/-</sup> (7 male, 7 female), 11 APP<sup>+/-</sup> (7 male, 4 female), and 8 APP<sup>+/+</sup> (5 male, 3 female) at 13–15 months old. MMP-3 mRNA expression was examined at all timepoints and protein levels were examined at the latest time point. Animal experimentation was performed in accordance with the McGill Animal Care Committee.

McGill-R-Thy1-APP rats express human Amyloid Precursor Protein (APP) with the double Swedish and the Indiana mutations under the Thy1 promoter, and recapitulate key features of amyloid pathology in AD (Leon et al., 2010). Amyloid-beta accumulates early in hippocampal and cortical neurons (Iulita et al., 2014b; Leon et al., 2010). As the amyloid pathology progresses with age, extracellular plaques start to develop progressively. The first plaques are observed in the subiculum at 6–9 months of age in homozygotes (Leon et al., 2010) and at 10–12 months of age in heterozygotes, and later spread through anatomically connected regions (Hegglund et al., 2015). Cognitive deficits are apparent at 3 months of age in homozygotes and at 6 months of age in heterozygotes, and worsen as the pathology progresses (Iulita et al., 2014b; Leon et al., 2010).

### **Neurochemical analysis of human frontal cortex samples**

We acquired dorsolateral/medial prefrontal cortex tissue (Brodmann's areas 9 and 46; areas previously shown to demonstrate significant proNGF accumulation/mNGF depletion (Iulita et al., 2014c; Pentz et al., 2020)) from 33 aged individuals with no cognitive impairment (NCI) and minimal cortical Aβ deposition (2 times the Standard Error of the Mean (SEM) under the mean of the MCI/AD groups; referred as Low-Amyloid-NCI; LA-NCI), 21 individuals with NCI but AD/MCI-like Aβ deposition (within 2 SEM of the mean of the MCI/AD groups; referred as High-Amyloid-No Cognitive Impairment; HA-NCI), 19 persons with a diagnosis of Mild Cognitive Impairment (MCI), and 24 patients with a clinical diagnosis of Alzheimer's dementia from the Religious Orders Study (ROS) Rush Alzheimer's Disease Center, Chicago, IL). All participants enroll without known dementia

and agree to annual detailed clinical evaluation and brain donation. The study was approved by an Institutional Review Board of Rush University Medical Center. All participants signed an informed consent, Anatomic Gift Act, and a repository consent to allow their biospecimens and data to be repurposed. ROS resources can be requested at <https://www.radc.rush.edu>. Diagnoses of Alzheimer's dementia were made according to the National Institute of Neurologic and Communicative Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann et al., 1984). As part of the Religious Orders Study (ROS) (Bennett et al., 2012), these individuals had undergone extensive cognitive testing including the Modified Mental State Exam (MMSE) and a series of 21 cognitive tests (episodic memory, semantic memory, working memory, perceptual orientation, and processing speed) recorded before death, expressed as z-scores and summarized as the Global Cognitive Score (GCS).

The ROS also entails a uniform *post-mortem* evaluation of brain's neuropathology. An overall measure of A $\beta$  deposition is taken by the average percent area of 6E10 A $\beta$  immunoreactivity in the CA1, subiculum, angular gyrus, entorhinal cortex, superior frontal cortex, dorsolateral prefrontal cortex, inferior temporal cortex, anterior cingulate cortex and calcarine cortex by image analysis (Bennett et al., 2006). An overall measure of tau tangle pathology was calculated by the stereological assessment of AT8 immunoreactivity, averaged across the entorhinal cortex, CA1, superior frontal cortex, mid frontal cortex, inferior temporal cortex, angular gyrus, cingulate gyrus, and calcarine cortex (Bennett et al., 2006). Patients were diagnosed with MCI if determined by a blinded neuropsychologist to have a cognitive impairment while deemed by a clinician to not meet criteria for dementia. The demographic characteristics of the study groups are illustrated in Table 1.

Tissue samples were prepared for Western blotting by sequential sonic and physical homogenization in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Nonidet p-40, 0.1% SDS, 0.1% sodium deoxycholate) containing protease inhibitors (cComplete Mini Protease Inhibitor tablets, Roche Diagnostics, Mannheim, Germany), following the protocol described above.

### qPCR for MMP-3 and TIMP-1 in human and rat brain tissue

RNA was isolated from cortex tissue samples matching those described above using the RNeasy RNA Isolation Kit (QIAGEN, Hilden, Germany). The integrity of the mRNA samples was analyzed using the NANODROP to ensure no contamination by DNA or protein. The samples were then reverse transcribed to rtDNA using iScript Reverse Transcription Supermix (Bio-Rad, Hercules, California) in a T3 Thermocycler (Biometra, Göttingen, Germany). Samples were combined with forward and reverse primers for MMP-3 (human forward: 5'-GTAGAAGGCACAATATGG-3', human reverse: 5'-ACTCTATGTGACAAGGTG-3'; rat forward: 5'-GTGCTCATCCTACCCATTGCAT-3', rat reverse: 5'-TTTGACAACAGGGCTACTGTCCCTT-3'; Integrated DNA Technologies, Coralville, USA), as well as SsoAdvanced CyberGreen Supermix (Bio-Rad). The samples were then analyzed in triplicate using CFX Connect Real Time PCR Machine (Bio-Rad, Hercules, USA), and the average  $2^{-Cq}$  values of each group were compared. The Cq values for each sample were normalized by the Cq value for GAPDH (5'-

CCCCACTTGATTTTGGAGGGA-3' forward, 5'-AGGGCTGCTTTTAACTCTGGT-3' reverse) in each sample. Results are presented as fold change from the mean of the WT group. Average GAPDH Cq values did not differ significantly between groups.

## Statistics

D'Agostino-Pearson Omnibus Normality Tests were used to determine the normality of groups, and group means were compared with one-way ANOVAs and Bonferroni post-hoc tests or Kruskal-Wallis tests and Dunn's post-hoc corrections. A two-way ANOVA with Bonferroni post-hoc tests was used to assess main effects of sex and interactions of sex and diagnosis. Differences in demographic characteristics were examined using ANOVA or Chi-square tests as appropriate (see Table 1). GraphPad Prism 5 was used for all statistics described above (GRAPHPAD Software, San Diego, California, USA). All bar graphs indicate the mean  $\pm$  Standard Error of the Mean (SEM).

Correlations were examined in R (R Project for Statistical Computing, Vienna, Austria) using multiple linear regression, with age and PMI included as cofactors and partial  $r^2$  and  $p$  values reported independently within each sex. Illustrative scatterplots were prepared with GraphPad PRISM 5. All values were normalized to the mean of the LA-NCI group and represented as a fold-change from that mean.

## Results

### MMP-3 as a mature NGF-degrading protease both *in vitro* and *in vivo*

Past work demonstrating that MMP-9 and MMP-3 target sequences overlap (Eckhard et al., 2016), that MMP-3 can be released in response to cholinergic stimulation (De Couto et al., 2009; Reina et al., 2011), and that MMP-3 was elevated in AD CSF where it correlated to cognitive decline (Hanzel et al., 2014) led us to consider the possibility that MMP-3 could be an mNGF-degrading protease like MMP-9 in the context of the proposed NGF metabolic pathway (Bruno and Cuello, 2006). To test this possibility, we co-incubated increasing doses of MMP-3 with different mNGF concentrations *in vitro*. The addition of recombinant MMP-3 overnight at 37 C led to a dose-dependent decrease in mNGF immunoreactivity ( $n = 4$ ; Figure 1A). Indeed, 14.8 mM MMP-3 was enough to almost completely abrogate mNGF immunoreactivity in that time ( $n = 4$ , Figure 1B).

Given these results, we next investigated if such mNGF proteolytic activity is observed in the mature CNS *in vivo*. For this, we resorted to inject stereotaxically in the medial dorsal hippocampus, in separate experimental animals, either MMP-3 or UK356618, a specific and well-validated inhibitor of MMP-3 (Artham et al., 2019), with the vehicle solution injected on the contralateral side in each condition. After 12–16 hours we trisected each hippocampus for comparisons of injected and non-injected segments (see Figure 2A for a schematic of injections).

Interestingly, MMP-3 injected into the medial dorsal hippocampus led to a near-total depletion of mNGF immunoreactivity in this segment in comparison both to the non-injected anterior and posterior segments, as well as each contralateral segment (One-way ANOVA, overall  $F = 16.00$ ,  $p < 0.001$ ; Bonferroni *post-hoc* tests for ipsilateral medial vs all others, all

$t > 5.80$ , all  $p < 0.001$ ; Figure 2B), while the saline-injected medial contralateral side did not differ from the non-injected anterior and posterior segments (*post-hoc* tests for contralateral medial vs anterior/posterior, all  $t < 1.30$ , all  $p > 0.05$ ; Figure 2B). Conversely, 2  $\mu\text{l}$  of 0.5  $\mu\text{M}$  UK356618 (a selective MMP-3 inhibitor) led to an approximate 25% increase in mNGF immunoreactivity in the injected segment versus all others (One-way ANOVA, overall  $F = 4.84$ ,  $p < 0.01$ ; Bonferroni *post-hoc* tests for ipsilateral medial vs all others, all  $t > 2.80$ , all  $p < 0.05$ ; ipsilateral medial versus ipsilateral anterior,  $t = 4.64$ ,  $p < 0.001$ ; Figure 2C). No such effect was observed for the injection of DMSO on the contralateral side (*post-hoc* tests for contralateral medial vs contralateral anterior/posterior, all  $t < 0.80$ , all  $p > 0.05$ ; Figure 2C). In contrast, no effect of the application MMP-3 or UK356618 was observed on hippocampal proNGF levels (Supplementary Figure 1A and 1B respectively).

### **MMP-3 is released from the rat cerebral cortex cells in response to cholinergic stimulation**

The NGF metabolic pathway is co-released from cholinergic target neurons in response to cholinergic stimulation, in a manner dependent on intracellular (but not extracellular) calcium (Bruno and Cuello, 2006). Having shown that MMP-3 has a role in mNGF degradation, we proceeded to investigate whether MMP-3 might be released in this same way, compatible with co-release as a part of the NGF metabolic pathway. Following (Bruno and Cuello, 2006), diced rat cortical tissue was superfused and stimulated with 100 nM carbachol or 50 mM KCl. Under both conditions, an activity-dependent release of MMP-3 was observed that peaked after 5 minutes of carbachol stimulation and returned to baseline by 30 minutes in superfusate samples (Figure 3A–B; average values of three replicates). The non-specific depolarizing agent KCl (50 mM) acted equivalently to carbachol (Supplementary Figure 2A and 2B), indicating that cholinergic stimulation suffices to elicit the release of MMP-3. The release of MMP-3 protein, as it is the case for other members of the NGF metabolic pathway (Bruno and Cuello, 2006), was shown to depend on intracellular but not extracellular calcium, as the cell permanent calcium chelator BAPTA-AM, but not the cell-impermeant BAPTA, abrogated MMP-3 release in response to carbachol (Figure 3A vs. 3B) or KCl (Supplementary Figure 1A vs. 1B).

### **MMP-3 levels are increased in a transgenic rat model of Alzheimer's-like amyloid pathology**

To examine whether MMP-3 levels are progressively altered in the presence of AD pathology, we assessed the transcription of MMP-3 mRNA at three time-points throughout the lifetime of the McGill-R-Thy1-APP transgenic rat model of Alzheimer's-like amyloid pathology.

At 3 months, which coincides with the first detection of cognitive impairments and proNGF upregulation in homozygotes (Iulita et al., 2014b; Iulita et al., 2017), MMP-3 mRNA was increased in homozygous transgenic rats ( $n = 7$ ) compared to wildtypes ( $n = 7$ ) and heterozygotes ( $n = 6$ ; One-way ANOVA:  $F = 6.33$ ,  $p = 0.009$ ; Bonferroni *post hoc* tests vs. homozygotes:  $t = 2.94$ ,  $p < 0.05$  vs. wildtype,  $t = 3.18$ ,  $p < 0.05$  vs. homozygotes; Figure 4A). Both homozygotes ( $n = 6$ ) and heterozygotes ( $n = 6$ ) expressed higher MMP-3 mRNA at 6 months compared to wildtypes ( $n = 7$ ; One-way ANOVA:  $F = 8.47$ ,  $p = 0.003$ ; Bonferroni *post hoc* tests vs. wildtype:  $t = 3.87$ ,  $p < 0.01$  vs. heterozygotes,  $t = 3.03$ ,  $p < 0.05$



vs. homozygotes; Figure 4B), a time-point prior to plaque pathology but with widespread intracellular A $\beta$  accumulation in both heterozygotes and homozygotes (Iulita et al., 2014b). At 13–15 months plaque pathology has been shown in homozygotes but not heterozygotes (Iulita et al., 2014b); nevertheless, homozygotes (n = 8) and heterozygotes (n = 11) both expressed higher MMP-3 mRNA at 13–15 months compared to wildtypes (n = 14) but did not differ from one another (One-way ANOVA:  $F = 12.34$ ,  $p < 0.0001$ ; Bonferroni *post hoc* tests:  $t = 4.95$ ,  $p < 0.001$  vs. heterozygotes,  $t = 2.93$ ,  $p < 0.05$  vs. homozygotes; heterozygotes vs. homozygotes,  $p < 0.05$ ; Figure 4C). These results were validated by demonstrating the same effect with protein by Western blotting: higher MMP-3 integrated optic density (IOD) in homozygotes (n = 6) and heterozygotes (n = 6) than wildtypes (n = 7) at 13–15 months (One-way ANOVA:  $F = 8.27$ ,  $p = 0.003$ ; Bonferroni *post hoc* tests vs. wildtype:  $t = 2.48$ ,  $p < 0.05$  vs. heterozygotes,  $t = 4.03$ ,  $p < 0.01$  vs. homozygotes; Figure 4D).

### Alterations of MMP-3 levels in the human cerebral cortex through the continuum of AD pathology

In order to understand how MMP-3 levels might change in the context of progressive AD pathology, we assessed the expression and levels of MMP-3 and proMMP-3 by qPCR and Western blot in dorsomedial prefrontal cortex samples from individuals with no cognitive impairment and minimal levels of amyloid deposition (LA-NCI; n = 33), in individuals with no cognitive impairment and MCI/AD-like high amyloid deposition (HA-NCI; n = 21), in Mild Cognitive Impairment (MCI; n = 19) and in Alzheimer's disease (AD; n = 24), cases. Demographic characteristics of the study cohort are displayed in Table 1; no association was found between MMP-3 levels and age, years of education, post-mortem interval, or APOE4 frequency.

We found that MMP-3 mRNA was elevated in HA-NCI, MCI, and AD cases as compared to LA-NCI individuals (Kruskal-Wallis test:  $H = 46.44$ ,  $p < 0.0001$ ; *Dunn's post-hoc tests vs. LA-NCI*: RS (Rank Sum) =  $-24.87$  vs. HA-NCI, RS =  $-44.53$  vs. MCI, and RS  $-44.27$  vs. AD; Figure 5A). The MMP-3 precursor, proMMP-3, was also elevated in HA-NCI, MCI, and AD (Kruskal-Wallis test:  $H = 20.15$ ,  $p = 0.0002$ ; *Dunn's post-hoc tests vs. LA-NCI*: RS =  $-20.31$  vs. HA-NCI, RS =  $-30.63$  vs. MCI, and RS  $-27.55$  vs. AD; Figure 5B). Mature MMP-3 protein IOD followed the same pattern, with higher levels in HA-NCI, MCI, and AD (One-way ANOVA:  $F = 16.17$ ,  $p < 0.0001$ ; *Bonferroni post-hoc tests vs. LA-NCI*:  $t = 2.563$  vs. HA-NCI,  $t = 5.595$  vs. MCI, and RS  $5.942$  vs. AD; Figure 5C).

**Sex-specific differences in MMP-3 protein levels**—Since we had previously observed higher expression of MMP-3 protein in the plasma of males compared to females (Iulita et al., 2019; Iulita et al., 2016), we investigated potential sex differences in CNS MMP-3 protein levels. A two-way, sex by diagnosis ANOVA demonstrated main effects (MEs) of diagnosis and sex, with *post-hoc* tests revealing significant differences by sex in the LA-NCI but not in other groups (Two-way ANOVA,  $F_{\text{DIAGNOSIS}} = 25.74$ ,  $p < 0.001$ ,  $F_{\text{SEX}} = 15.19$ ,  $p < 0.001$ ,  $F_{\text{INTERACTION}} = 0.04$ ,  $p > 0.05$ ; Bonferroni *post hoc* tests: sex within LA-NCI  $t = 2.72$ ,  $p < 0.05$ ; sex within HA-NCI  $t = 1.99$ ,  $p > 0.05$ , sex within MCI  $t = 1.45$ ,  $p > 0.05$ , sex within HA-NCI  $t = 1.54$ ,  $p > 0.05$ ; Figure 5D).

**Sex-specific relationship between MMP-3 protein expression and AD neuropathology or cognitive scores**

—We were then interested in understanding whether MMP-3 might be associated with cognitive and neuropathological changes associated with AD. Considering only the persons with no cognitive impairment (NCI), we correlated levels of MMP-3 protein to two cognitive measures: the Mini-Mental State Exam (MMSE), a commonly used dementia screen, and the Global Cognitive Score (GCS), a multi-domain composite of cognitive tests used to broadly assess cognitive function. Additionally, we correlated MMP-3 protein levels to measures of A $\beta$  deposition and tau tangle density over multiple cortical regions. We found that higher MMP-3 protein was associated with lower scores on both the MMSE and the CGS, as well as higher burden of A $\beta$  and more tau tangles (Table 2).

Since we observed a sexually dimorphic expression of MMP-3 most pronounced at stages preceding cognitive impairment in this and previous studies (Iulita et al., 2019; Iulita et al., 2016) we were interested in whether sex would influence the associations between MMP-3 protein and the cognitive and pathological changes associated with AD at preclinical stages. Intriguingly, we observed significant associations between increased MMP-3 protein levels and lower scores on both the MMSE and GCS in males with NCI (n = 27) that were not found in females (n = 27; Table 2; Supplementary Figure 3A and B). Similarly, MMP-3 protein correlated with A $\beta$  burden and tau tangle density in NCI males, while in females, only an association with A $\beta$  burden was observed (Table 2; Supplementary Figure 3C and D).

**Sex-specific relationship between MMP-3 protein expression, proNGF and TIMP-1**

—Given that we propose a role for MMP-3 in the metabolism of mNGF, we were interested to observe whether MMP-3 protein levels would correlate to levels of mNGF, the NGF precursor proNGF, and the CNS inhibitor of MMP-3, TIMP-1 (given by mRNA expression levels). Interestingly, males exhibited a moderate negative correlation between MMP-3 protein levels and mNGF protein levels (Table 2; Supplementary Figure 4A), a strong positive correlation between MMP-3 levels and proNGF (Table 2; Supplementary Figure 4B), and a weak correlation between TIMP1 mRNA levels and MMP-3 (Table 2; Supplementary Figure 4C) in the dorsomedial prefrontal cortex, while none of these were observed in females (Table 2; Supplementary Figure 4A–C). As before, while all these correlations were evident in the overall population (Table 2), they appear to have been driven by the males in the population.

**Discussion**

In this study, we demonstrate for the first time a role for MMP-3 in the proteolysis of mNGF and, furthermore, its involvement in the NGF dysmetabolism unleashed by AD pathology (Figure 6), predominantly by amyloid accumulation. This proposition is supported by the fact that MMP-3 protein, its precursor, and mRNA transcripts were found elevated over the course of human AD, reaching near-maximum levels prior to the onset of dementia, in parallel with accumulating AD pathology. We also demonstrate that the expression of MMP-3 in the human frontal cortex exhibits dimorphism by sex, with males having consistently more MMP-3 immunoreactivity. Additionally, MMP-3 appears to correlate to

measures of pathology in males in a way that is generally not apparent in females, though the underlying mechanism is unclear.

### **MMP-3 and AD: genetics and neuropathology**

The involvement of MMP-3 in AD pathogenesis or symptomology is supported by limited genetic evidence. An interaction between APOE  $\epsilon 4$  status and MMP-3–1171- 5A/6A allele status (associated with promoter activity) that influences risk of AD was found in three cohorts (Helbecque et al., 2007; Saarela et al., 2004), though it was absent in a fourth (Reitz et al., 2008); an underlying mechanism was never elucidated, though several studies suggested that they might synergistically contribute to risk of vascular dysfunction and indeed, an interactions between MMP-3 and APOE  $\epsilon 4$  status were found to contribute to pathology only in patients with heart disease in one study (Helbecque et al., 2007). The link between MMP-3 genetic variants and AD risk would suggest a mechanistic role of this protease in AD pathology/symptomology, which may in part explain the findings of our study.

While this is, to our knowledge, the first study to investigate brain MMP-3 levels in MCI and in cognitively normal people with AD-like neuropathology, it has been previously demonstrated that MMP-3 protein levels are increased in AD, particularly in white matter and in proximity to amyloid plaques (Yoshiyama et al., 2000). That observation is in line with our finding of correlations between amyloid deposition and MMP-3 levels in cognitively normal aged persons as well as with previous studies showing elevated MMP-3 secretion in mixed hippocampal cultures after treatment with A $\beta$  peptides (Deb and Gottschall, 1996), suggesting a direct link between amyloid deposition and MMP-3 accumulation. While a role of MMP3 in degrading A $\beta$  peptides has been proposed (White et al., 2006) it is unlikely that metallo-proteases have a significant effect in diminishing the amyloid pathology given that most studies have shown amyloid pathology and metalloproteases levels correlate positively, if at all (Baig et al., 2008).

While A $\beta$  was shown to induce MMP-3 expression, previous studies demonstrated that MMP-3 could in turn reduce tau oligomer aggregation by cleaving tau in core repeat sequences (Nübling et al., 2012). In the present investigation, we observed significant associations between MMP-3 and tau deposition in males, but not in females, providing partial support for this hypothesis, though it is still unclear how these mechanisms could be modulated by sex.

A $\beta$  pathology and increased MMP-3 levels are further linked by the present demonstration that MMP-3 transcription is upregulated in the McGill-R-Thy1-APP homozygotic transgenic rats modelling the AD-like amyloid pathology at 3 months and in heterozygotes at 6 months. It is worth noting that at 3 months of age, McGill-R-Thy1-APP homozygotes first display cognitive deficits (Leon et al., 2010) and start revealing proNGF accumulation (Iulita et al., 2017). Furthermore, no difference in MMP-3 levels were observed at the 13–15-month timepoint between McGill-R-Thy1-APP homozygotes and heterozygotes, when homozygotes display extensive plaque pathology and heterozygotes remain plaque-free. This suggests that the MMP-3 upregulation might be primarily driven by soluble A $\beta$  and

therefore explain why MMP-3 and plaque deposition only correlated in the early disease state.

### **MMP-3 and AD: the bigger picture**

In this study, we highlighted a novel role for MMP-3 as a mNGF protease, participating in the regulation of trophic support to BFCNs. However, other important roles have been proposed for MMP-3 in AD. MMP-3 is a critical mediator of inflammation, where it can serve to activate microglia (Kim et al., 2007; Kim et al., 2005; Lee et al., 2010; Walker and Rosenberg, 2009), facilitate peripheral immune cell invasion (Gurney et al., 2006), and cleave inactive precursors to produce active pro-inflammatory cytokines (Schönbeck et al., 1998; Woo et al., 2008); since MMP-3 is itself inducible by cytokine signalling (Borghaei et al., 2004; Sinfield et al., 2013; Tamai et al., 1995; Wang et al., 2014) and free oxygen radicals (Liu and Rosenberg, 2005), it may participate in a pathological positive feedback loop. MMP-3 has also postulated to play roles in AD-associated processes such as neurite outgrowth (Cua et al., 2013; Gonthier et al., 2007; Pizzi and Crowe, 2006), long-term potentiation (Brzdak et al., 2017; Brzdak et al., 2019; Meighan et al., 2006; Wiera et al., 2017), myelin turnover/damage (D'Souza et al., 2002; D'Souza and Moscarello, 2006; Hoyos et al., 2016; Opendakker and Van Damme, 1994; Rosenberg et al., 2001), apoptosis, and blood-brain barrier integrity (Gasche et al., 2001; Gurney et al., 2006; Okada et al., 1987). Indeed, the upregulation of MMP-3 and other MMPs has been proposed as the mechanism by which A $\beta$  damages the blood-brain barrier (Brkic et al., 2015) and MMP-3 induction may be a conserved response to cell stress (Choi et al., 2008; Kim et al., 2010). Therefore, MMP-3 participation on NGF dysmetabolism should also be taken into consideration in the context of other MMP-3-elicited processes in AD pathology.

### **MMP-3 as a candidate AD biomarker**

Previous studies have implicated MMP-3 as a potential early biomarker of AD. Several studies have shown that MMP-3 is increased in plasma (Horstmann et al., 2010; Iulita et al., 2019; Peng et al., 2015) and CSF (Hanzel et al., 2014; Horstmann et al., 2010) from patients with symptomatic AD, as well as in people with Down syndrome who manifest AD (Iulita et al., 2016). CSF MMP-3 has also been shown to be associated with core biomarkers of AD, showing positive correlations with CSF total-tau and phospho-tau (Hanzel et al., 2014; Stomrud et al., 2010b), and curiously, positive correlations with A $\beta$ 42 were found in one study (Mlekusch and Humpel, 2009). MMP-3 activity in plasma (Peng et al., 2015) and levels in CSF (Hanzel et al., 2014) have been demonstrated to negatively correlate with MMSE scores. The current study, showing negative correlations between MMSE/GCS performance and brain MMP-3 levels demonstrates that in the previous studies, biofluid MMP-3 levels are reflecting CNS MMP-3.

While biofluid MMP-3 has yet to be systematically analyzed at the early stages of AD, plasma levels of MMP-3 were found elevated in patients with subjective memory impairments and mild cognitive impairment as compared to healthy controls (Iulita et al., 2019). Furthermore, healthy aged individuals with AD risk factors revealed higher levels of CSF MMP-3 than healthy aged individuals without AD risk factors (Stomrud et al., 2010b). In Down syndrome, a condition characterized by a lifelong and inexorably progressive

amyloidosis that culminates in clinical AD (Lott and Head, 2019), plasma MMP-3 is elevated and increasing even prior to the onset of overt dementia (Iulita et al., 2016). An exploratory study also suggested an A $\beta$ -associated effect of MMP-3 on brain atrophy in cognitively normal aged individuals (Mattsson et al., 2014). Our present report provides new grounds supporting the above clinical observations, as we demonstrated an increase of MMP-3, associations with cognitive ability and associations with A $\beta$  deposition prior to the onset of cognitive symptoms in AD. Considering the newly described role of MMP-3 in mNGF proteolysis, MMP-3 could perhaps serve as part of a panel that could rarify AD diagnosis by adding information about NGF dysmetabolism and cholinergic decline, and could perhaps enhance precision medicine by providing sex-specific indications with regards to pathology and/or cognition.

### A link between MMP-3 and NGF metabolism

The discovery of the NGF metabolic pathway, describing the maturation and subsequent degradation of NGF in the CNS (Bruno and Cuello 2006), permitted a better understanding of trophic support to basal forebrain cholinergic neurons in health and disease (Bruno and Cuello, 2006; Cuello et al., 2010; Cuello et al., 2019; Iulita and Cuello, 2014). As these neurons are fully dependent on endogenous mNGF for the maintenance of their cholinergic phenotype and functions (Cuello, 1993; Debeir et al., 1998), we entertain the hypothesis that NGF dysmetabolism in AD best explains both cholinergic atrophy as well as the brain's build-up of proNGF together with the depletion of mNGF observable in this condition, even prior to the onset of overt dementia (Bruno et al., 2009b; Pentz et al., 2020). This neurotrophic failure results from impaired conversion of proNGF into mNGF and the exacerbated degradation of already diminished mNGF (see Figure 6 for schematic overview).

The NGF metabolic pathway has been proposed as a source of new biomarkers and therapeutic targets for the cholinergic deficit in AD (Counts et al., 2016; Cuello et al., 2019; Iulita et al., 2019). In this report, we offer a fuller understanding of NGF dysmetabolism by demonstrating the *in vitro* and *in vivo* ability of an additional metallo-proteinase, MMP-3, to degrade mNGF. While it is difficult to estimate the exact correspondence of our experiments to physiological levels of MMP-3/mNGF, it is likely that the excess of MMP-3 we used was appropriate, as mNGF is present at vanishingly low concentrations in the human brain (Fahnestock et al., 2001). The absence of proNGF cleavage is likely a product of the change in shape induced by the presence of the prodomain which may shield the vulnerable moiety (Trabjerg et al., 2017; Yan et al., 2019). We further demonstrated that MMP-3 is secreted in an activity-dependent manner, in response to nonspecific and cholinergic stimulation in a manner dependant on intracellular calcium, similarly to other members of the NGF metabolic pathway. We further validate the relevance of this finding by demonstrating that MMP-3 elevation occurs early in the pathogenesis of AD. These findings are in line with the thorough investigation of other molecular members of the NGF metabolic pathways across the continuum of AD (Pentz et al., 2020). Importantly, this report provides evidence that higher MMP-3 correlates to lower mNGF and higher proNGF in cognitively normal aged adults.

## Sex differences in MMP-3 expression in AD

In this study we report that MMP-3 levels are lower in females, and that correlations between MMP-3 and the preclinical neuropathological and cognitive symptoms of AD are most—sometimes only—observable in males. Accordingly, previous studies have indicated higher biofluid levels of MMP-3 in males (Almodóvar et al., 2014; Arends et al., 2011; Iulita et al., 2019; Iulita et al., 2016; Yamanaka et al., 2000) and have shown differential response of MMP-3 to disease between sexes (Arends et al., 2011; Ribbens et al., 2002), supporting a differential biological regulation of MMP-3 in males and females. Interestingly, a previous retrospective cohort study had demonstrated that lower MMP-3 levels correlated with poorer performance on the MoCA and Delated Recall tests in females with AD and MCI but not in males (Iulita et al., 2019). While a model integrating both of these studies—better correlations between brain MMP-3 and pathology in males and better correlations between plasma MMP-3 and cognitive decline in females—remains to be elucidated in the context of increased female risk of AD, it is possible that MMP-3 is responding to pathology in males in a way that is not paralleled in females; this could serve a protective function with regards to amyloidosis that unfortunately exacerbates NGF dysmetabolism. The lack of a correlation between MMP-3 and mNGF in females may also indicate that MMP-9 plays a more important role in females than in males in terms of NGF metabolism. This may also illuminate certain differences in cholinergic dysfunction and treatment in AD, with some data indicating a better response to cholinesterase inhibitors in males (Giacobini and Pepeu, 2018). These results also highlight the critical importance of considering the role of sex in AD pathology, and particularly the risks of generalizing findings in males, who have historically been disproportionately investigated, to the entire human population—as recently highlighted by the Women’s Brain Project (Ferretti et al., 2018; Ferretti et al., 2020).

## Conclusion

These studies would indicate that, independently of previously established roles, MMP-3 additionally plays a physiological role in regulating the availability of trophic support to basal forebrain cholinergic neurons by degrading mature NGF. We also demonstrate that MMP-3 is upregulated in the dorsomedial prefrontal cortex throughout preclinical and clinical stages of AD. We further show that these levels are higher in males, and that MMP-3 levels in cognitively normal aged males (but not females) consistently correlate to cognitive decline, AD neuropathology, and NGF dysmetabolism. These results support future preclinical studies investigating MMP-3 as an early biomarker and as a potential therapeutic target in AD with adequate consideration of sex as a factor.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

The authors first and foremost express gratitude to the ROS participants and their families for their immense contributions, advancing science towards new treatments for AD. We also thank the RUSH Medical Center team, who accomplished all cognitive and neuropathological testing and scoring.

The authors would also like to acknowledge Dr. M. Beatriz Bistué Millón (Universidad Católica de Cuyo, Argentina) for helpful discussions about the NGF proteolytic assay and release experiments.

ACC and his team acknowledge support from Canadian Institutes of Health Research (CIHR) grant 20129MOP-285643-PT-CFAA-22808 and a Women Gender Sex and Dementia grant # 244230 from the Canadian Consortium of Neurodegeneration in Aging (CCNA). He is the holder of the McGill University Charles E. Frosst/Merck Chair in Pharmacology and a Member of the CCNA. DAB and his team were supported by grants P30AG10161 and R01AG15819 from the NIA. RP was the recipient of a CIHR Doctoral Award and a Returning Student Fellowship from the McGill Integrated Program in Neuroscience. MFI acknowledges support from a Bourse Postdoctorale from the Jérôme Lejeune Foundation, France (ongoing) and from the Fonds de Recherche du Québec Santé (2015–2019). There was no input or approval from any funding body with regards to the study design, execution, analysis, interpretation, or presentation.

## Abbreviations

<b>A<math>\beta</math></b>	Amyloid beta
<b>AD</b>	Alzheimer's disease
<b>APP</b>	Amyloid Precursor Protein
<b>BFC</b>	Basal Forebrain Cholinergic
<b>BFCN</b>	Basal Forebrain Cholinergic Neuron
<b>CNS</b>	Central nervous system
<b>GCS</b>	Global cognitive scores
<b>HA-NCI</b>	High Amyloid, No Cognitive Impairment
<b>INT</b>	Interaction
<b>IOD</b>	Integrated Optical Density
<b>LA-NCI</b>	Low Amyloid, No Cognitive Impairment
<b>MCI</b>	Mild Cognitive Impairment
<b>ME</b>	Main Effect
<b>MMP-3/9</b>	Matrix Metalloproteinase-3/9
<b>MMSE</b>	Mini-mental state examination
<b>mNGF</b>	mature Nerve Growth Factor
<b>NCI</b>	No Cognitive Impairment
<b>NGF</b>	Nerve Growth Factor
<b>proMMP-3</b>	precursor to MMP-3
<b>proNGF</b>	the precursor to Nerve Growth Factor
<b>SEM</b>	Standard Error of the Mean
<b>TIMP1</b>	Tissue Inhibitor of Metalloproteinases-1

<b>tPA</b>	tissue Plasminogen Activator
<b>WT</b>	wild-type

## References

- Almodóvar R, et al., 2014 Association of biomarkers of inflammation, cartilage and bone turnover with gender, disease activity, radiological damage and sacroiliitis by magnetic resonance imaging in patients with early spondyloarthritis. *Clinical rheumatology*. 33, 237–241. [PubMed: 23917391]
- Arends S, et al., 2011 Serum MMP-3 level as a biomarker for monitoring and predicting response to etanercept treatment in ankylosing spondylitis. 38, 1644–1650.
- Artham S, et al., 2019 Endothelial stromelysin1 regulation by the forkhead box-O transcription factors is crucial in the exudative phase of acute lung injury. *Pharmacological research*. 141, 249–263. [PubMed: 30611853]
- Baig S, et al., 2008 MMP-2,-3 and-9 levels and activity are not related to A $\beta$  load in the frontal cortex in Alzheimer's disease. *Neuropathology and applied neurobiology*. 34, 205–215. [PubMed: 17971072]
- Bartus RT, et al., 1982 The cholinergic hypothesis of geriatric memory dysfunction. *Science*. 217, 408–414. [PubMed: 7046051]
- Bennett D, et al., 2006 Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*. 66, 1837–1844. [PubMed: 16801647]
- Bennett D, et al., 2012 Overview and findings from the religious orders study. *Current Alzheimer Research*. 9, 628–645. [PubMed: 22471860]
- Borghaei RC, et al., 2004 NF- $\kappa$ B binds to a polymorphic repressor element in the MMP-3 promoter. *Biochemical biophysical research communications*. 316, 182–188. [PubMed: 15003528]
- Brkic M, et al., 2015 Amyloid  $\beta$  oligomers disrupt blood–CSF barrier integrity by activating matrix metalloproteinases. *Journal of Neuroscience*. 35, 12766–12778. [PubMed: 26377465]
- Bruno MA, Cuello AC, 2006 Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. *Proceedings of the National Academy of Sciences*. 103, 6735–6740.
- Bruno MA, et al., 2009a Amyloid  $\beta$ -induced nerve growth factor dysmetabolism in Alzheimer disease. *Journal of Neuropathology & Experimental Neurology*. 68, 857–869. [PubMed: 19606067]
- Bruno MA, et al., 2009b Increased Matrix Metalloproteinase-9 Activity in Mild Cognitive Impairment. *Journal of neuropathology and experimental neurology*. 68, 1309. [PubMed: 19915485]
- Brzdak P, et al., 2017 Matrix metalloprotease 3 activity supports hippocampal EPSP-to-spike plasticity following patterned neuronal activity via the regulation of NMDAR function and calcium flux. *Molecular neurobiology*. 54, 804–816. [PubMed: 27351676]
- Brzdak P, et al., 2019 Synaptic potentiation at basal and apical dendrites of hippocampal pyramidal neurons involves activation of a distinct set of extracellular and intracellular molecular cues. *Cerebral Cortex*. 29, 283–304. [PubMed: 29228131]
- Choi DH, et al., 2008 A novel intracellular role of matrix metalloproteinase-3 during apoptosis of dopaminergic cells. *Journal of neurochemistry*. 106, 405–415. [PubMed: 18397366]
- Côté, S., et al., 1993 *Immunocytochemistry II*. NY: John Wiley & Sons.
- Counts S, et al., 2016 Cerebrospinal fluid proNGF: a putative biomarker for early Alzheimer's disease. *Current Alzheimer Research*. 13, 800–808. [PubMed: 26825093]
- Cua RC, et al., 2013 Overcoming neurite-inhibitory chondroitin sulfate proteoglycans in the astrocyte matrix. *Glia*. 61, 972–984. [PubMed: 23554135]
- Cuello AC, 1993 Trophic responses of forebrain cholinergic neurons. *Progress in brain research*. 98, 265–265. [PubMed: 8248516]
- Cuello AC, et al., 2010 Cholinergic involvement in Alzheimer's disease. A link with NGF maturation and degradation. *Journal of molecular neuroscience*. 40, 230–235. [PubMed: 19680822]
- Cuello AC, et al., 2019 The brain NGF metabolic pathway in health and in Alzheimer's pathology. *Frontiers in neuroscience*. 13, 62. [PubMed: 30809111]



- D'Souza CA, et al., 2002 The up-regulation of stromelysin-1 (MMP-3) in a spontaneously demyelinating transgenic mouse precedes onset of disease. *Journal of Biological Chemistry*. 277, 13589–13596.
- D'Souza CA, Moscarello MA, 2006 Differences in susceptibility of MBP charge isomers to digestion by stromelysin-1 (MMP-3) and release of an immunodominant epitope. *Neurochemical research*. 31, 1045–1054. [PubMed: 16871440]
- De Couto PA, et al., 2009 Cholinoceptor modulation on nitric oxide regulates prostaglandin E (2) and metalloproteinase-3 production in experimentally induced inflammation of rat dental pulp. *Journal of endodontics*. 35, 529. [PubMed: 19345799]
- Deb S, Gottschall PE, 1996 Increased production of matrix metalloproteinases in enriched astrocyte and mixed hippocampal cultures treated with  $\beta$ -amyloid peptides. *Journal of neurochemistry*. 66, 1641–1647. [PubMed: 8627321]
- Debeir T, et al., 1998 TrkA antagonists decrease NGF-induced ChAT activity in vitro and modulate cholinergic synaptic number in vivo. *Journal of Physiology-Paris*. 92, 205–208.
- Drachman DA, Leavitt J, 1974 Human memory and the cholinergic system: a relationship to aging? *Archives of neurology*. 30, 113–121. [PubMed: 4359364]
- Eckhard U, et al., 2016 Active site specificity profiling of the matrix metalloproteinase family: Proteomic identification of 4300 cleavage sites by nine MMPs explored with structural and synthetic peptide cleavage analyses. *Matrix biology*. 49, 37–60. [PubMed: 26407638]
- Everitt BJ, Robbins TW, 1997 Central cholinergic systems and cognition. *Annual review of psychology*. 48, 649–684.
- Fahnestock M, et al., 2001 The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease. *Molecular Cellular Neuroscience*. 18, 210–220. [PubMed: 11520181]
- Ferretti M, et al., 2018 Women's Brain Project and the Alzheimer Precision Medicine Initiative: Sex differences in Alzheimer disease-the gateway to precision medicine. *Nat Rev Neurol*. 14, 457–469. [PubMed: 29985474]
- Ferretti MT, et al., 2020 Sex and gender differences in Alzheimer's disease: current challenges and implications for clinical practice: Position paper of the Dementia and Cognitive Disorders Panel of the European Academy of Neurology. *European Journal of Neurology*. 27, 928–943. [PubMed: 32056347]
- Garofalo L, et al., 1992 Nerve growth factor-induced synaptogenesis and hypertrophy of cortical cholinergic terminals. *Proceedings of the National Academy of Sciences*. 89, 2639–2643.
- Gasche Y, et al., 2001 Matrix metalloproteinase inhibition prevents oxidative stress-associated blood–brain barrier disruption after transient focal cerebral ischemia. *Journal of Cerebral Blood Flow Metabolism*. 21, 1393–1400. [PubMed: 11740200]
- Giacobini E, Pepeu G, 2018 Sex and gender differences in the brain cholinergic system and in the response to therapy of Alzheimer disease with cholinesterase inhibitors. *Current Alzheimer Research*. 15, 1077–1084. [PubMed: 29895246]
- Gonthier B, et al., 2007 Functional interaction between matrix metalloproteinase-3 and semaphorin-3C during cortical axonal growth and guidance. *Cerebral Cortex*. 17, 1712–1721. [PubMed: 17021275]
- Gurney KJ, et al., 2006 Blood–brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. *Neurobiology of disease*. 23, 87–96. [PubMed: 16624562]
- Hanzel CE, et al., 2014 Analysis of matrix metallo-proteases and the plasminogen system in mild cognitive impairment and Alzheimer's disease cerebrospinal fluid. *Journal of Alzheimer's disease*. 40, 667–678.
- Hasselmo ME, Sarter M, 2011 Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology*. 36, 52–73. [PubMed: 20668433]
- Hefti F, 1986 Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *Journal of Neuroscience*. 6, 2155–2162. [PubMed: 3746405]
- Heggland I, et al., 2015 Stereological estimation of neuron number and plaque load in the hippocampal region of a transgenic rat model of Alzheimer's disease. *European Journal of Neuroscience*. 41, 1245–1262.

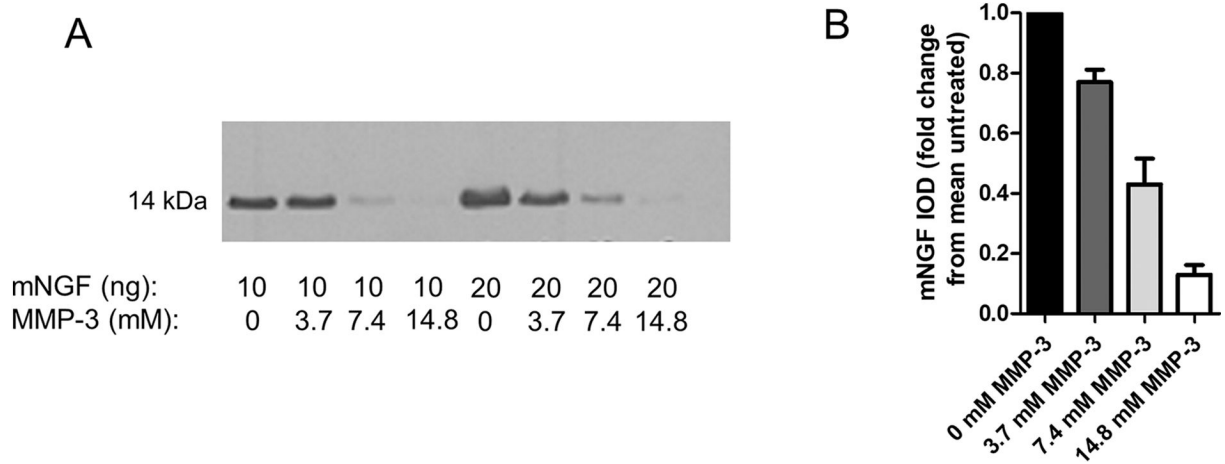
- Helbecque N, et al., 2007 Impact of the matrix metalloproteinase MMP-3 on dementia. *Neurobiology of aging*. 28, 1215–1220. [PubMed: 16822591]
- Horstmann S, et al., 2010 Matrix metalloproteinases in peripheral blood and cerebrospinal fluid in patients with Alzheimer's disease. *International psychogeriatrics*. 22, 966–972. [PubMed: 20561382]
- Hoyos HC, et al., The role of galectin-3: from oligodendroglial differentiation and myelination to demyelination and remyelination processes in a cuprizone-induced demyelination model. *Glial Cells in Health and Disease of the CNS*. Springer, 2016, pp. 311–332.
- Iulita MF, et al., 2014a Intracellular A $\beta$  pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study. *Acta neuropathologica communications*. 2, 1. [PubMed: 24383468]
- Iulita MF, et al., 2014b Intracellular A $\beta$  pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study. *Acta neuropathologica communications*. 2, 61. [PubMed: 24903713]
- Iulita MF, et al., 2017 Differential deregulation of NGF and BDNF neurotrophins in a transgenic rat model of Alzheimer's disease. *Neurobiol Dis*. 108, 307–323. [PubMed: 28865749]
- Iulita MF, Cuello AC, 2014 Nerve growth factor metabolic dysfunction in Alzheimer's disease and Down syndrome. *Trends in pharmacological sciences*. 35, 338–348. [PubMed: 24962069]
- Iulita MF, et al., 2014c Nerve growth factor metabolic dysfunction in Down's syndrome brains. *Brain*. 137, 860–872. [PubMed: 24519975]
- Iulita MF, et al., 2019 Identification and Preliminary Validation of a Plasma Profile Associated with Cognitive Decline in Dementia and At-Risk Individuals: A Retrospective Cohort Analysis. *Journal of Alzheimer's Disease*. 67, 327–341.
- Iulita MF, et al., 2016 An inflammatory and trophic disconnect biomarker profile revealed in Down syndrome plasma: Relation to cognitive decline and longitudinal evaluation. *Alzheimer's & Dementia*.
- Kim E-M, et al., 2010 Matrix metalloproteinase-3 is increased and participates in neuronal apoptotic signaling downstream of caspase-12 during endoplasmic reticulum stress. *Journal of Biological Chemistry*. 285, 16444–16452.
- Kim YS, et al., 2007 A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglial activation. *The FASEB Journal*. 21, 179–187. [PubMed: 17116747]
- Kim YS, et al., 2005 Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. *Journal of Neuroscience*. 25, 3701–3711. [PubMed: 15814801]
- Lee E-J, et al., 2010 a-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. *The Journal of Immunology*. 185, 615–623. [PubMed: 20511551]
- Leon WC, et al., 2010 A novel transgenic rat model with a full Alzheimer's-like amyloid pathology displays pre-plaque intracellular amyloid- $\beta$ -associated cognitive impairment. *Journal of Alzheimer's Disease*. 20, 113–126.
- Liu KJ, Rosenberg GA, 2005 Matrix metalloproteinases and free radicals in cerebral ischemia. *Free Radical Biology*. 39, 71–80.
- Locke S, et al., 2020 Peripheral and central nervous system alterations in a rat model of inflammatory arthritis. *Pain*.
- Lott IT, Head E, 2019 Dementia in Down syndrome: unique insights for Alzheimer disease research. *Nature Reviews Neurology*. 15, 135–147. [PubMed: 30733618]
- Mattsson N, et al., 2014 Effects of cerebrospinal fluid proteins on brain atrophy rates in cognitively healthy older adults. 35, 614–622.
- McKhann G, et al., 1984 Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group\* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 34, 939–939. [PubMed: 6610841]
- Meighan SE, et al., 2006 Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. *Journal of neurochemistry*. 96, 1227–1241. [PubMed: 16464240]

- Mesulam M, 2004 The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learning Memory*. 11, 43–49. [PubMed: 14747516]
- Mlekusch R, Humpel C, 2009 Matrix metalloproteinases-2 and-3 are reduced in cerebrospinal fluid with low beta-amyloid1–42 levels. *Neuroscience letters*. 466, 135–138. [PubMed: 19786072]
- Nübling G, et al., 2012 Limited cleavage of tau with matrix-metalloproteinase MMP-9, but not MMP-3, enhances tau oligomer formation. *Experimental neurology*. 237, 470–476. [PubMed: 22890115]
- Ogata Y, et al., 1992 Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. *Journal of Biological Chemistry*. 267, 3581–3584.
- Okada Y, et al., 1987 Matrix metalloproteinases 1, 2, and 3 from rheumatoid synovial cells are sufficient to destroy joints. *The Journal of rheumatology*. 14, 41–42. [PubMed: 3305938]
- Opdenakker G, Van Damme J, 1994 Cytokine-regulated proteases in autoimmune diseases. *Immunology today*. 15, 103–107. [PubMed: 8172641]
- Paxinos G, Watson C, 2006 *The rat brain in stereotaxic coordinates: hard cover edition*. Elsevier.
- Pearson R, et al., 1983 Persistence of cholinergic neurons in the basal nucleus in a brain with senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for choline acetyltransferase. *Brain research*. 289, 375–379. [PubMed: 6362777]
- Peng M, et al., 2015 Plasma gelsolin and matrix metalloproteinase 3 as potential biomarkers for Alzheimer disease. *Neuroscience letters*. 595, 116–121. [PubMed: 25864780]
- Pentz R, et al., 2020 The human brain NGF metabolic pathway is impaired in the pre-clinical and clinical continuum of Alzheimers disease. *Molecular Psychiatry*. 1–15.
- Pizzi MA, Crowe MJ, 2006 Transplantation of fibroblasts that overexpress matrix metalloproteinase-3 into the site of spinal cord injury in rats. *Journal of neurotrauma*. 23, 1750–1765. [PubMed: 17184186]
- Reina S, et al., 2011 Anti-M3 muscarinic cholinergic autoantibodies from patients with primary Sjögren's syndrome trigger production of matrix metalloproteinase-3 (MMP-3) and prostaglandin E2 (PGE2) from the submandibular glands. *archives of oral biology*. 56, 413–420. [PubMed: 21371688]
- Reitz C, et al., 2008 Matrix metalloproteinase 3 haplotypes and dementia and Alzheimer's disease: the Rotterdam Study. *Neurobiology of aging*. 29, 874–881. [PubMed: 17316907]
- Ribbens C, et al., 2002 Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment. *Annals of the Rheumatic Diseases*. 61, 161–166. [PubMed: 11796404]
- Rosenberg GA, et al., 2001 White matter damage is associated with matrix metalloproteinases in vascular dementia. *Stroke*. 32, 1162–1166. [PubMed: 11340226]
- Saarela MS, et al., 2004 Interaction between matrix metalloproteinase 3 and the  $\epsilon 4$  allele of apolipoprotein E increases the risk of Alzheimer's disease in Finns. *Neuroscience letters*. 367, 336–339. [PubMed: 15337261]
- Schönbeck U, et al., 1998 Generation of biologically active IL-1 $\beta$  by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1 $\beta$  processing. *The Journal of Immunology*. 161, 3340–3346. [PubMed: 9759850]
- Sinfield JK, et al., 2013 p38 MAPK alpha mediates cytokine-induced IL-6 and MMP-3 expression in human cardiac fibroblasts. *Biochemical biophysical research communications*. 430, 419–424. [PubMed: 23206705]
- Stomrud E, et al., 2010a Alterations of matrix metalloproteinases in the healthy elderly with increased risk of prodromal Alzheimer's disease. *Alzheimer's research & therapy*. 2, 20.
- Stomrud E, et al., 2010b Alterations of matrix metalloproteinases in the healthy elderly with increased risk of prodromal Alzheimer's disease. 2, 20.
- Tamai K, et al., 1995 Interferon- $\gamma$  coordinately upregulates matrix metalloprotease (MMP)-1 and MMP-3, but not tissue inhibitor of metalloproteases (TIMP), expression in cultured keratinocytes. *Journal of investigative dermatology*. 104, 384–390.
- Trabjerg E, et al., 2017 Conformational characterization of nerve growth factor- $\beta$  reveals that its regulatory pro-part domain stabilizes three loop regions in its mature part. *Journal of Biological Chemistry*. 292, 16665–16676.

- Walker EJ, Rosenberg GA, 2009 TIMP-3 and MMP-3 contribute to delayed inflammation and hippocampal neuronal death following global ischemia. *Experimental neurology*. 216, 122–131. [PubMed: 19111539]
- Wang X, et al., 2014 Tumor necrosis factor- $\alpha$ -and interleukin-1 $\beta$ -dependent matrix metalloproteinase-3 expression in nucleus pulposus cells requires cooperative signaling via syndecan 4 and mitogen-activated protein kinase-NF- $\kappa$ B axis: implications in inflammatory disc disease. *The American journal of pathology*. 184, 2560–2572. [PubMed: 25063530]
- White AR, et al., 2006 Degradation of the Alzheimer disease amyloid  $\beta$ -peptide by metal-dependent upregulation of metalloprotease activity. *Journal of Biological Chemistry*. 281, 17670–17680.
- Whitehouse PJ, et al., 1982 Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science*. 215, 1237–1239. [PubMed: 7058341]
- Wiera G, et al., 2017 Mechanisms of NMDA receptor-and voltage-gated L-type calcium channel-dependent hippocampal LTP critically rely on proteolysis that is mediated by distinct metalloproteinases. *Journal of Neuroscience*. 37, 1240–1256. [PubMed: 28069922]
- Woo MS, et al., 2008 Inhibition of MMP-3 or-9 suppresses lipopolysaccharide-induced expression of proinflammatory cytokines and iNOS in microglia. *Journal of neurochemistry*. 106, 770–780. [PubMed: 18419763]
- Yamanaka H, et al., 2000 Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. *Arthritis*. 43, 852–858.
- Yan R, et al., 2019 The structure of the Pro-domain of mouse proNGF in contact with the NGF domain. *Structure*. 27, 78–89. e3. [PubMed: 30393051]
- Yoshiyama Y, et al., 2000 Selective distribution of matrix metalloproteinase-3 (MMP-3) in Alzheimer's disease brain. *Acta neuropathologica*. 99, 91–95. [PubMed: 10672313]

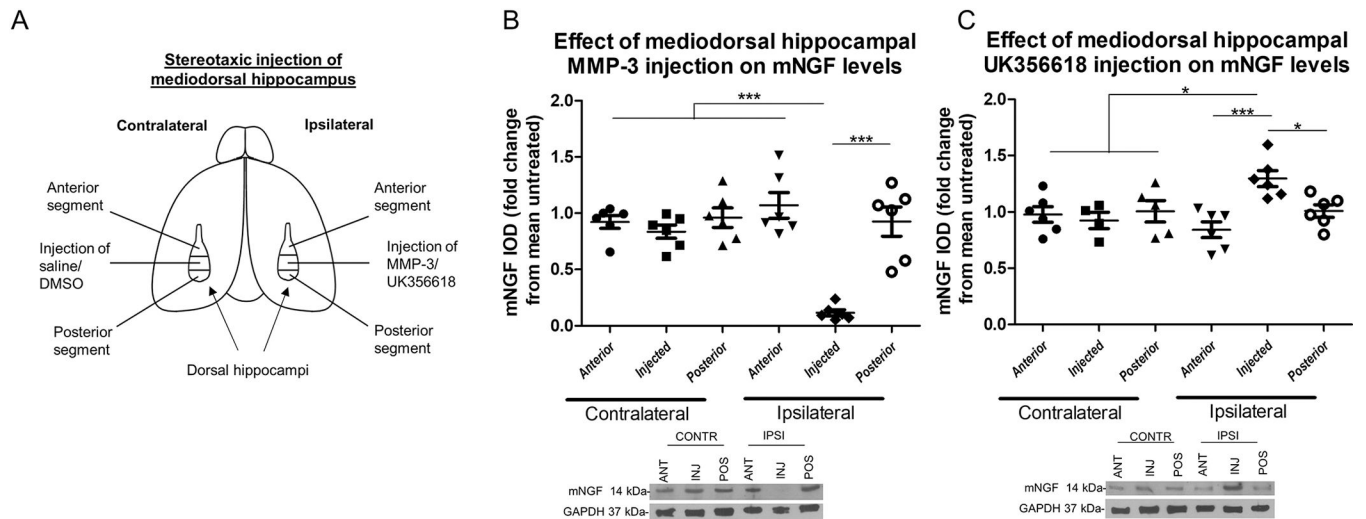
### Highlights

- MMP-3 degraded mature NGF *in vitro* and the inhibition of MMP-3 *in vivo* enhanced brain levels of mature NGF while the infusion of MMP-3 abrogated them
- MMP-3 is released from brain cortex in response to cholinergic stimulation
- MMP-3 mRNA and protein are increased in a rat model of AD-like amyloidosis and in human MCI/AD prefrontal cortex
- MMP-3 is increased in cognitively normal aged individuals with high brain amyloid
- MMP-3 protein in human frontal cortex is higher in males than females
- MMP-3 protein correlates to cognitive scores and measures of AD neuropathology in cognitively healthy aged males but not in females



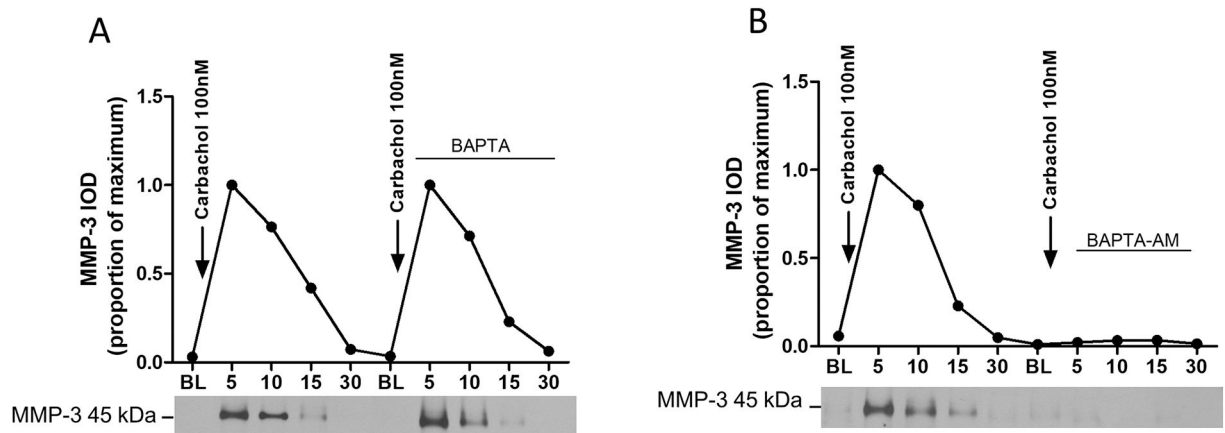
**Figure 1. Proteolysis of mature NGF by MMP-3 *in vitro*.**

**A:** Representative Western blot showing that the coincubation of 10 or 20 nanograms of recombinant human mature NGF with 0, 3.7, 7.4, or 14.8 mM of active recombinant human MMP-3 overnight at 37°C led to progressive reductions in NGF immunoreactivity. **B:** Quantitative analysis of the dose-dependent degradation of mature NGF incubated with 0 to 3.7 mM MMP-3 as in A, expressed as a proportion of the quantified immunoreactivity of the untreated sample within a set and averaged between the 10 and 20 ng conditions. Bars indicate the average plus the SEM. mNGF = mature Nerve Growth Factor, MMP-3 = Matrix Metalloproteinase-3, IOD = Integrated Optic Density.



**Figure 2. MMP-3 contributes to the proteolysis of mature NGF *in vivo*.**

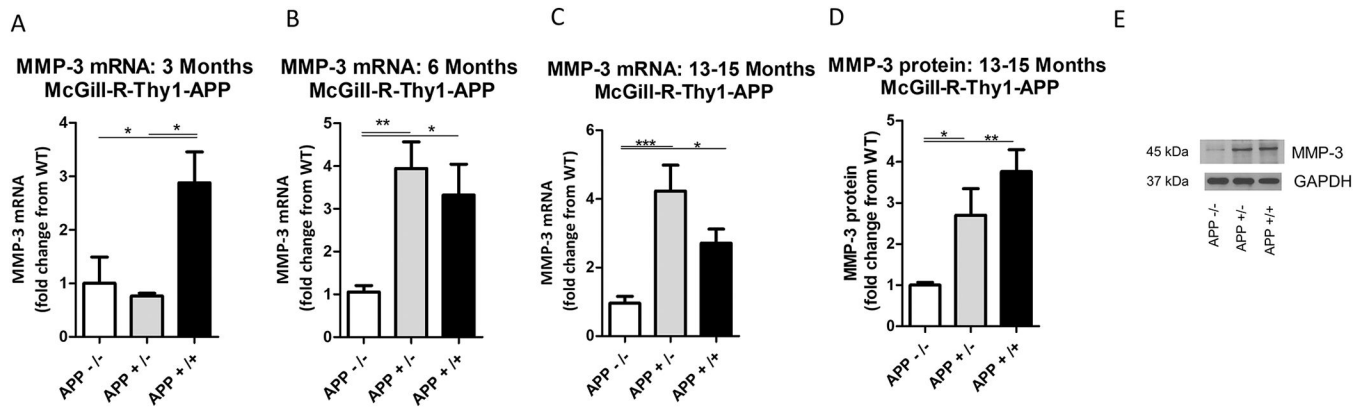
**A:** to test the ability of MMP-3 to degrade mNGF *in vivo*, either MMP-3 or the selective MMP-3 inhibitor, UK356618 was stereotaxically injected into the medial hippocampus on one side, while the contralateral medial segment received an injection of vehicle solution. The injected medial segments were compared to each other and to the non-injected anterior and posterior segments on each side. **B:** The injection of 2.0  $\mu$ l of MMP-3 in saline solution (1.0  $\mu$ g/ $\mu$ l) into the medial hippocampus led to a near-total abrogation of mNGF immunoreactivity in this segment in comparison both to the anterior and posterior ipsilateral segments, as well as to each contralateral segment. **C:** The injection of 2.0  $\mu$ l of 0.5  $\mu$ M UK356618, prepared in DMSO, led to a ~25% increase in mNGF immunoreactivity in the injected segment when compared to the ipsilateral anterior and posterior segments, as well as to all contralateral segments. No such effect was observed for the injection of DMSO on the contralateral side. Data is displayed as scatterplots showing individual values and the mean  $\pm$  SEM. All groups comprised 6 rats, 3 males and 3 females. MMP-3 = Matrix Metalloproteinase-3, NGF = Nerve Growth Factor, IOD = Integrated Optic Density.



**Figure 3. Activity-dependent release of MMP-3 in response to cholinergic stimulation in superfused ex-vivo rat brain cortical tissue.**

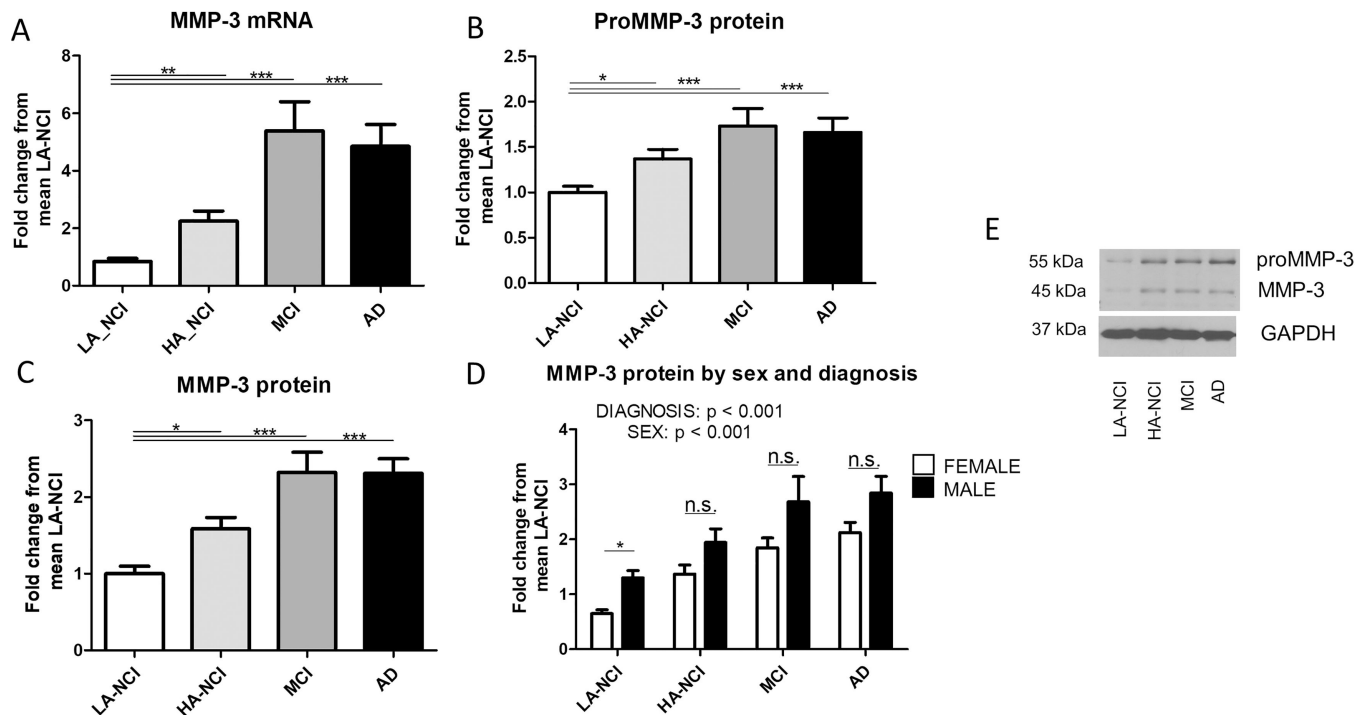
MMP-3 levels in ex vivo rat cerebral cortex superfusates are elevated 5 minutes after stimulation with the cholinergic agonist carbachol, decrease by roughly 25% after 10 minutes, decrease by 50–75% after 15 minutes, and return to baseline by 30 minutes. These responses are not affected by the cell-impermeant calcium chelator BAPTA (**Panel A**) but are abrogated by the cell-permeant calcium chelator BAPTA-AM (**Panel B**). Samples from a single experiment were run on the same blot and values are expressed as proportions of the maximum immunoreactivity observed; each dot represents the average of three experiments. MMP-3: Matrix Metalloproteinase-3, IOD: Integrated Optical Density.





**Figure 4. Analysis of MMP-3 expression ( $2^{-Cq}$ ) in cerebral cortex from the McGill-R-Thy1-APP transgenic rat model of AD-like amyloidosis.**

**A:** At 3 months of age, MMP-3 mRNA was upregulated in homozygous transgenics compared to heterozygotes and wild-types, which were not different one from another. **B/C:** At both the six and the 13–15 month time-points, MMP-3 mRNA in both the heterozygous and homozygous transgenics were increased relative to the wild-types. **D:** Confirming that these transcriptional differences have relevance for protein levels, we showed that MMP-3 protein was also elevated in heterozygous and homozygous transgenics relative to the wild-types at the 13–15 months timepoint. **E:** Illustrative Western Blot for MMP-3 in wildtype, hemizygous, and homozygous McGill-R-Thy1-APP rats, with GAPDH for reference. For all figures, stars indicate the significance of Bonferonni *post-hoc* tests vs. APP -/- in a 4x1 ANOVA: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  Data represents Cq or Integrated Optic Densities, with bars representing mean values and error bars representing the SEM. MMP-3 = Matrix Metalloproteinase-3.

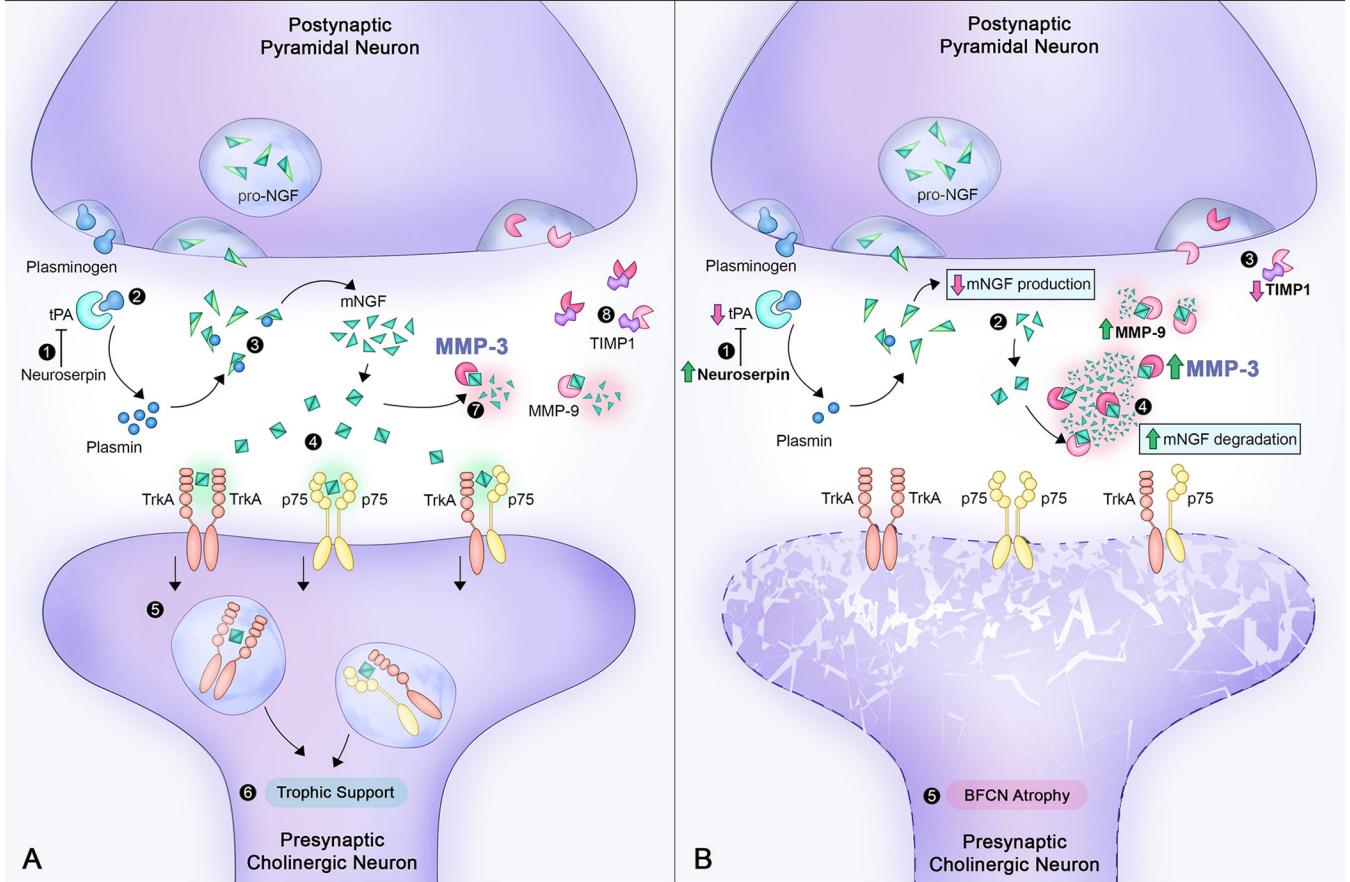


**Figure 5. Analysis of MMP-3 expression in the continuum of human Alzheimer's disease.**

(A) Levels of MMP-3 mRNA, (B) proMMP-3 and (C) MMP-3 protein were assessed in dorsolateral/medial prefrontal cortex samples from individuals with no cognitive impairment and low amyloid deposition (LA-NCI; n = 33), individuals with no cognitive impairment and AD/MCI-like levels of amyloid deposition (HA-NCI; n = 21), individuals with mild cognitive impairment (MCI; n = 19) and patients with AD dementia (AD; n = 24). MMP-3 mRNA (A), proMMP-3 protein (B), and mature MMP-3 protein (C) were all increased in AD and MCI, as well as in HA-NCI individuals compared to the LA-NCI group. D: A significant main effect of sex was observed, with males having higher levels of mature MMP-3 protein across all clinical classifications, but only demonstrating post-hoc significance vs. females in LA-NCI individuals. E: An illustrative Western Blot for proMMP-3 and MMP-3 in LA-NCI, HA-NCI, MCI, and AD, with GAPDH for reference. For A-C, stars indicate the significance of Bonferonni *post-hoc* tests vs. LA-NCI in a 4x1 ANOVA: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, while in D, they indicate post-hoc Bonferonni tests for male vs. female within clinical groups in a 4x2 ANOVA; the stars indicate equivalent significance. Panel A represents Cq values, while panels B-D represent Integrated Optic Density values, all expressed as a fold change from the LA-NCI group. All bars represent mean values with errors bars representing the SEM. MMP-3 = Matrix Metalloproteinase-3, proMMP-3: precursor to MMP-3

Non-Cognitively Impaired Brain

Non-Cognitively Impaired Brain with Early Alzheimer's Pathology



**Figure 6. Schematic of the NGF Metabolic Pathway in health in in the context of AD neuropathology.**

**A:** Under normal conditions, mNGF is derived from proNGF by plasmin (3), itself derived from plasminogen by tissue plasminogen activator (tPA) (2). tPA activity is controlled by neuroserpin, its endogenous inhibitor (1). MMP-9 and, we now show, MMP-3 degrade mNGF (7); these are regulated by the tissue inhibitor of metalloproteinases 1 (TIMP1) (8). Under normal conditions sufficient mNGF dimerizes and binds p75/TrkA receptor complexes (4). The activated receptors are internalized and retrogradely transported to the cell body where they ultimately exert their trophic effect (6). **B:** In the context of AD pathology, increased neuroserpin/decreased tPA (1) lead to reductions in local plasmin concentrations, reducing the maturation of proNGF to mNGF (2). Decreased TIMP1 (3) and increased MMP-3/MMP-9 lead to the excessive degradation of mNGF (4). As a result, trophic support to BFCNs is compromised (5). mNGF = mature Nerve Growth Factor, proNGF = precursor to NGF, tPA = tissue plasminogen activator, BFCN = basal forebrain cholinergic neuron, MMP-3/9 = matrix metalloproteinase 3/9, TIMP1 = tissue inhibitor of metalloproteinases 1

**Table 1:**

Demographic and neuropathological characteristics of ROS brain donors included in this study.

	LA NCI	HA-NCI	MCI	AD	p value (comparison between groups)	Association with MMP-3 in all samples
<b>n =</b>	33	21	19	24		
<b>Percent female</b>	45.5%	57.1%	57.9%	62.5%	0.26 (Chi- square test)	
<b>Age (years)</b>	79.2 ± 6.9	83.1 ± 8.7	83.9 ± 8.5	81.0 ± 8.2	0.16 (ANOVA)	r <sup>2</sup> = 0.03, p > 0.05 (regression)
<b>Post- mortem interval (hr)</b>	8.6 ± 6.1	8.5 ± 7.0	8.8 ± 5.6	6.3 ± 4.0	0.40 (ANOVA)	r <sup>2</sup> = 0.01, p > 0.05 (regression)
<b>Years of education</b>	18.2 ± 3.9	18.1 ± 2.6	18.2 ± 4.0	± 3.0	0.73 (ANOVA)	r <sup>2</sup> = 0.01, p > 0.05 (regression)
<b>Amyloid-β 42 percent area</b>	0.3 ± 0.4	3.7 ± 2.2	2.7 ± 2.4	3.8 ± 3.2	<0.0001 (ANOVA)	r <sup>2</sup> = 0.02, p > 0.05 (regression)
<b>NFT density/mm<sup>2</sup></b>	1.8 ± 2.0	3.9 ± 4.8	5.1 ± 6.7	10.0 ± 13.4	<0.0001 (ANOVA)	r <sup>2</sup> = 0.05, p < 0.05 (regression)
<b>BRAAK</b>	2.1 ± 1.3	3.3 ± 1.2	3.5 ± 0.8	3.7 ± 1.2	<0.0001 (ANOVA)	<b>r = 0.27, p &lt; 0.001 (correlation)</b>
<b>APOE ε4 allele frequency</b>	0.12	0.20	0.18	0.17	0.31 (ANOVA)	F <sub>APOE4</sub> = 0.33, p > 0.05; F <sub>INTERACTION</sub> = 0.70, p > 0.05
<b>MMSE (proportion correct)</b>	0.96 ± 0.04	0.95 ± 0.05	0.88 ± 0.10	0.45 ± 0.21	<0.0001 (ANOVA)	<b>r<sup>2</sup> = 0.16, p &lt; 0.0001 (regression)</b>
<b>Global Cognitive Score(z score)</b>	0.25 ± 0.33	0.13 ± 0.40	-0.22 ± 0.30	-2.59 ± 1.12	<0.0001 (ANOVA)	<b>r<sup>2</sup> = 0.19, p &lt; 0.0001 (regression)</b>

Data is presented as mean ± standard deviation. LA-NCI: low amyloid-no cognitive impairment, HA- NCI: high amyloid-no cognitive impairment, MCI: mild cognitive impairment, AD: Alzheimer's disease, NFT: neurofibrillary tangles, MMP-3: Matrix Metalloproteinase-3. The "p value" column gives the result of a one-way ANOVA or Chi-square test comparing the means of all four pathological group while the "Association" gives the results of regression/correlation between MMP-3 protein levels and demographic/pathological characteristics; the APOE4 association is the exception, showing the results of a two-way ANOVA between diagnosis and e4 carrier status in all samples.

**Table 2:**

Overall and Sex-stratified associations between MMP-3 and cognitive scores, neuropathological scores, and measures of NGF dysmetabolism in all cognitively normal aged individuals (NCI).

Covariate	Overall (n = 54)	Females (n = 27)	Males (n = 27)
MMSE (proportion correct)	$r^2 = \mathbf{0.15}$ $p = \mathbf{0.004}$	$r^2 = 0.02$ $p = 0.53$	$r^2 = \mathbf{0.32}$ $p = \mathbf{0.002}$
GCS (z-score)	$r^2 = \mathbf{0.11}$ $p = \mathbf{0.02}$	$r^2 = 0.03$ $p = 0.37$	$r^2 = \mathbf{0.29}$ $p = \mathbf{0.004}$
Amyloid- $\beta$ 42 percent area	$r^2 = \mathbf{0.18}$ $p = \mathbf{0.002}$	$r^2 = \mathbf{0.28}$ $p = \mathbf{0.005}$	$r^2 = \mathbf{0.28}$ $p = \mathbf{0.006}$
NFT density/mm <sup>2</sup>	$r^2 = \mathbf{0.15}$ $p = \mathbf{0.004}$	$r^2 = 0.02$ $p = 0.44$	$r^2 = \mathbf{0.29}$ $p = \mathbf{0.004}$
mNGF protein	$r^2 = \mathbf{0.04}$ $p = \mathbf{0.33}$	$r^2 = 0.01$ $p = 0.74$	$r^2 = \mathbf{0.37}$ $p = \mathbf{0.04}$
ProNGF protein	$r^2 = \mathbf{0.29}$ $p < \mathbf{0.0001}$	$r^2 = 0.04$ $p = 0.35$	$r^2 = \mathbf{0.55}$ $p < \mathbf{0.0001}$
TIMP1 mRNA	$r^2 = \mathbf{0.11}$ $p = \mathbf{0.02}$	$r^2 = 0.01$ $p = 0.70$	$r^2 = \mathbf{0.21}$ $p = \mathbf{0.03}$

Partial (age and PMI-corrected)  $r^2$  and p values are presented for each association. Significant associations ( $p < 0.05$ ) are bolded. NFT: neurofibrillary tangles, MMP-3: Matrix Metalloproteinase-3, proNGF: precursor to Nerve Growth Factor; TIMP1: Tissue Inhibitor of Metalloproteinases-1