JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2015, 66, 2, 309-315
www.jpp.krakow.pl

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IMPACT OF MATRIX METALLOPROTEINASE-9 OVEREXPRESSION ON SYNAPTIC EXCITATORY TRANSMISSION AND ITS PLASTICITY IN RAT CA3-CA1 HIPPOCAMPAL PATHWAY

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Metalloproteinases (MMPs) have been shown to play a crucial role in synaptic plasticity and cognitive processes. We recently reported that in the mossy fiber - CA3 hippocampal pathway, LTP maintenance required fine-tuned MMP-9 activity, as both MMP-9 excess and absence impaired LTP. Here we used acute brain slices from transgenic (TG) rats overexpressing MMP-9 to investigate the impact of excessive MMP-9 activity on the excitatory synaptic transmission in the CA3-CA1 projection. Using field potential recordings, we have demonstrated that MMP-9 overexpression increased the strength of basal synaptic transmission but had no effect on the short-term plasticity in comparison to the wild-type (WT) group. In attempt to shed light on mechanisms underlying this observation, miniature excitatory postsynaptic potentials (mEPSCs) were recorded from pyramidal CA1 neurons. We found that mEPSCs in the TG group had a significantly slower decaying phase than in WT but amplitudes and frequencies were similar. The lack of differences in mEPSC frequency and short-term plasticity between TG and WT groups suggests that MMP-9 overexpression effect on fEPSPs was mainly postsynaptic. Additionally, we have found that excess of MMP-9 in TG rats was associated with impaired late-phase of LTP in the considered pathway. It seems thus that augmented synaptic strength in TG rats occurred in expense of impaired long-term plasticity induced by tetanization. In conclusion, overexpression of MMP-9 leads to increase in the strength of basal excitatory synaptic transmission and impairs of LTP maintenance phase in the CA3-CA1 pathway *in vitro*.

Key words: *hippocampus, metalloproteinase, high frequency stimulation, long-term potentiation, miniature excitatory postsynaptic potentials, field excitatory postsynaptic potentials, synapse*

INTRODUCTION

Extracellular proteolysis is well known to reorganize the extracellular matrix (ECM) but it has been also found to trigger profound changes in function and morphology of neurons. These processes are initiated by degradation of ECM components and cell surface proteins. Several lines of evidence indicate that metalloproteinases (MMPs) play a crucial role in synapse remodeling and long-term functional synaptic plasticity (1-3). Expression and secretion of MMP-9 by hippocampal pyramidal neurons were found to accompany seizures, maintenance phase of long-term potentiation and memory acquisition (4-9).

Recent studies have demonstrated that synaptic MMP-9 dependent proteolysis regulated the efficacy of synaptic transmission and the morphology of dendritic spines. MMP-9 knockout mice exhibit impaired LTP in hippocampal mossy fiber - CA3 (mf-CA3) and CA3-CA1 projections (5, 10), decrease in experience-dependent plasticity in the barrel cortex (11) as well as deficits in hippocampus-dependent memory tested in context fear conditioning or novel object recognition paradigms (5, 12). Administration of MMP-9 protein to neuronal cultures decreases the number of mushroom-shaped dendritic spines, increases the number of filopodia and spine head protrusions (13, 14). Additionally, exogenous MMP-9 protease potentiates synaptic response with simultaneous spine expansion (5, 10, 15, 16). Recently, Michaluk *et al.* (17) have shown, in dissociated neuronal cultures, that excessive MMP-9 causes elongation and thinning of dendritic spines, which is accompanied by a prolongation of the miniature excitatory postsynaptic currents (mEPSCs) decaying phase, suggesting a 'juvenalization' of glutamatergic synapses. In our recent study we have provided evidence that fine-tuned level of MMP-9 activity is required for LTP consolidation in mf-CA3 pathway, as both absence and excess of MMP-9 impaired LTP (10). Among above described findings the most intriguing is that excessive MMP-9 disrupts LTP maintenance in the mf-CA3 pathway. In the present study we pursued this issue in the CA3-CA1 projection in transgenic rats constitutively overexpressing MMP-9 protease in neurons (18). We find that excess of MMP-9 in this model affects basal fEPSP (field excitatory postsynaptic potentials), the late phase of LTP and the time course of miniature EPSCs.

MATERIAL AND METHODS

Animals, hippocampal slices preparation and field potential recordings

Experiments were performed on 4–10 weeks old male Wistar rats and the transgenic (TG) strain overexpressing auto-active MMP-9 under the control of synapsin I promoter (18) in agreement with the European Union directive on animal experimentation. This model is characterized by at least 10 times more MMP-9 activity in hippocampal homogenates, as verified using gel gelatin zymography. Brain slices were prepared as described previously (10, 19). Shortly, 350 µm transverse slices were prepared in ice-cold buffer bubbled with 95% O_2/ 5% CO_2, which contained: (in mM) NaCl, 87; KCl, 2.5; $NaHCO_3$, 25; NaH_2PO_4, 1.25; $CaCl_2$, 0.5; $MgCl_2$, 7; glucose, 20; sucrose 75. Slices were kept in cutting solution for 20 minutes at 32°C then were transferred to artificial cerebrospinal fluid (ACSF) which contain (in mM): NaCl 125, $NaHCO_3$ 25, KCl 2.5, NaH_2PO_4 1.25, $CaCl_2$ 2.5, $MgCl_2$ 1, glucose 20, pH 7.4 for at least 2 h at room temperature. All substances were obtained from Sigma Aldrich, unless otherwise stated. Field potentials were recorded in submersion chamber in CA1 stratum radiatum at 30 ± 1°C. Stimulating electrode was placed in the Schaffer collateral-commissural pathway. Stimuli were delivered at 10 s intervals with current intensity yielding approximately 50% of maximal responses. In our previous report (20) we have presented detailed description of recording stability. To induce LTP, tetanic stimulation (100 pulses at 100 Hz, 4 trains with 10 s intervals) was applied. The amplitude or slope of fEPSPs responses was normalized to the pretetanus baseline.

Patch clamp and mEPSC analysis

Whole cell recordings were performed in ACSF with borosilicate patch pipettes (4-6 MΩ) and internal solution comprised (mM): potassium gluconate, 116; KCl, 6; NaCl, 2;

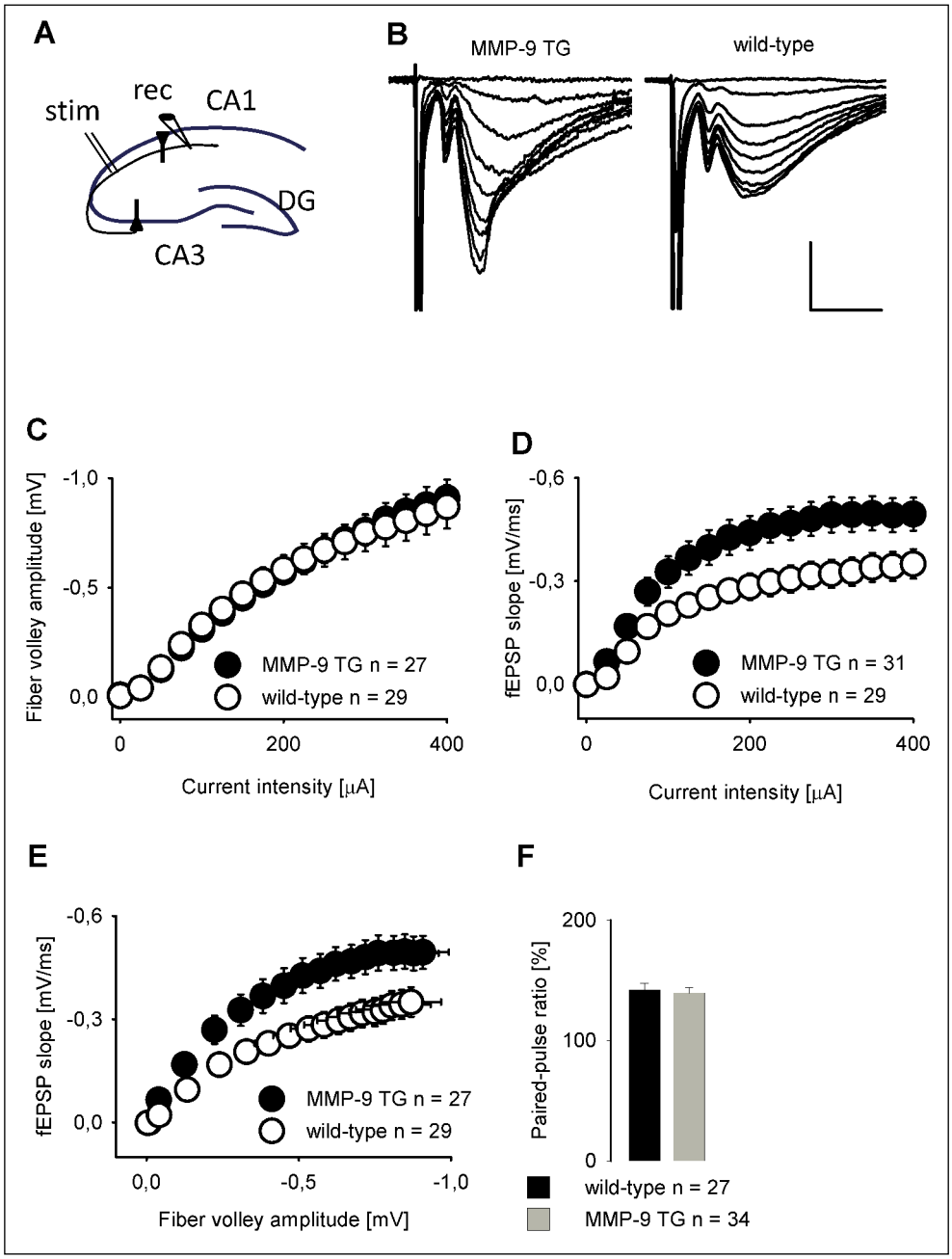

Fig. 1. The augmentation of synaptic transmission in MMP-9 overexpressing rats in CA3-CA1 pathway. A) Diagram of the hippocampus showing a CA1 pyramidal neuron receiving a synaptic input from a CA3 principal cell. The stimulating electrode (stim) was positioned in stratum radiatum. B) A typical example of I-O responses recorded in CA3-CA1 projection in MMP-9 TG and wild-type rats. Presented traces show fiber volley and synaptic response to increasing current intensities from 0 to 400 µA with 50 µA steps. Calibration: horizontal –5 ms, vertical –0.5 mV. C-E) Analysis of basal synaptic transmission in CA3-CA1 pathway in WT (open circles) and in MMP-9 overexpressing rats (filled circles). Mean amplitudes of fiber volley (C) and of fEPSP slopes (D) as a function of stimulation strength. E) I-O plot constructed as a relationship between fEPSP slopes and fiber volley amplitudes. Note the increased slope of basal fEPSPs in slices from MMP-9 overexpression rats without any effect on fiber volley amplitude. F) No significant difference in short-term plasticity assessed as the paired pulse ratio (interpulse interval 50 ms) was found between WT (black bars) and MMP-9 TG rats (grey bars).

HEPES, 20; EGTA, 0.5; MgATP, 4; NaGTP, 0.3; Na_2-phosphocreatine, 10; pH 7.25 (300 mOsm). CA1 neurons were voltage-clamped at –70 mV. For mEPSC recording, inhibitory postsynaptic potentials and action potentials were blocked by adding to the ACSF 10 μM gabazine and TTX (1 μM; LaToxan, France), respectively (21). The AMPAergic identity of mEPSCs was confirmed by blockade with DNQX (20 μM; Tocris, USA). The access resistance was monitored during the recordings and was in the range of 10–20 MΩ. Data analysis was restricted to pyramidal cells chosen based on common criteria of firing pattern, passive membrane properties described earlier (22) as well as morphological features of soma and apical dendrites. Kinetic analysis was performed on an averaged mEPSC from each neuron. Onset was quantified as 10–90% rise time (RT). Decay kinetics was fitted with single exponential function $y(t)=A \exp(-t/\tau_{decay})$, where A is the amplitude and τ_{decay} is the decay time constant.

Statistical analysis

Student's *t*-test was used for paired comparisons, two-way ANOVA was used to compare the fEPSP input-output relationships. Graphical data are shown as the mean ± standard error.

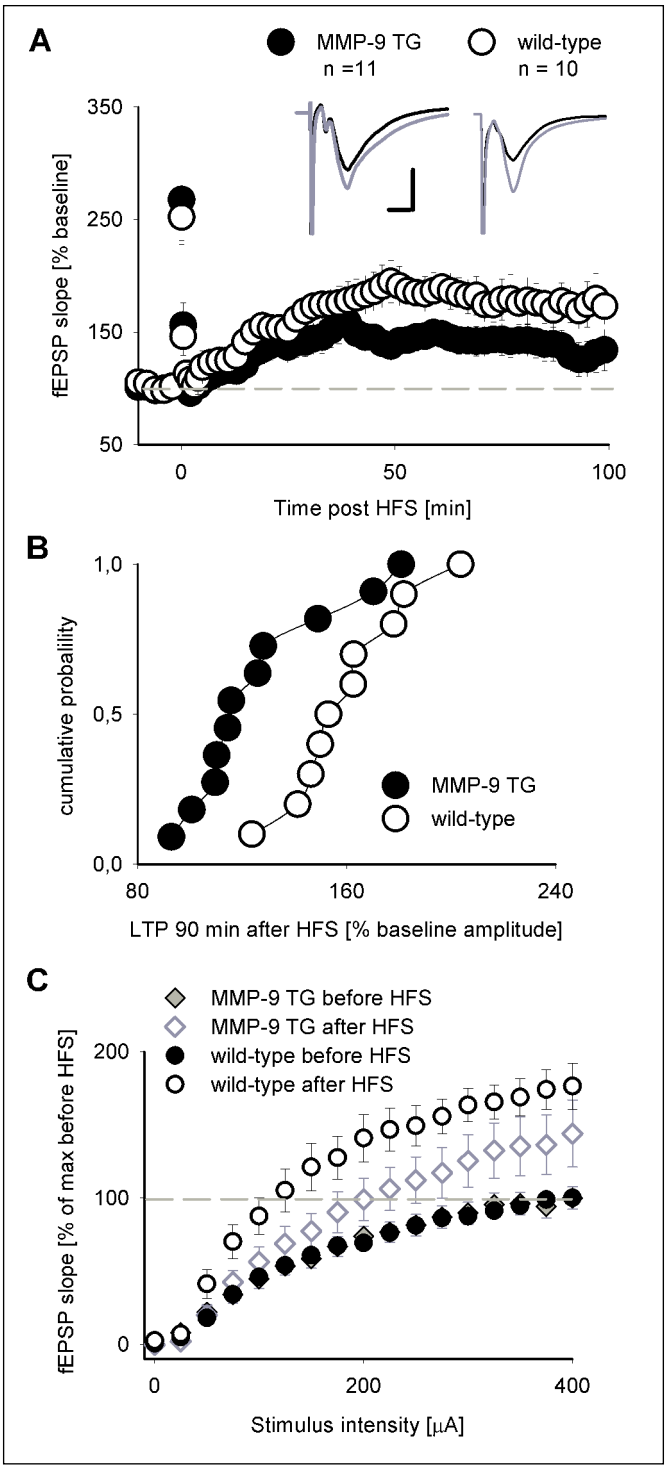

Fig. 2. The matrix metalloproteinase-9 (MMP-9) overexpression impairs LTP in CA3-CA1 projection. A) Late phase of HFS induced LTP is impaired in hippocampal slices from MMP-9 TG rats (filled circles) compared with that in wild-types (open circles). The LTP is induced at time 0. Insets present sample fEPSP recorded during the baseline period (black) and 100 min after HFS calibration: horizontal 5 ms, vertical 0.4 mV. B) Cumulative plot of CA3-CA1 LTP magnitude at 100 min after HFS. Note that values recorded in MMP-9 TG (black circles) show markedly less potentiation than in WT mice (open circles, $P < 0.05$). C) Average I-O relationships measured before (filled symbols) and 100 min after HFS (open symbols) in WT (diamonds, n = 10) and in MMP-9 TG slices (circles, n = 11).

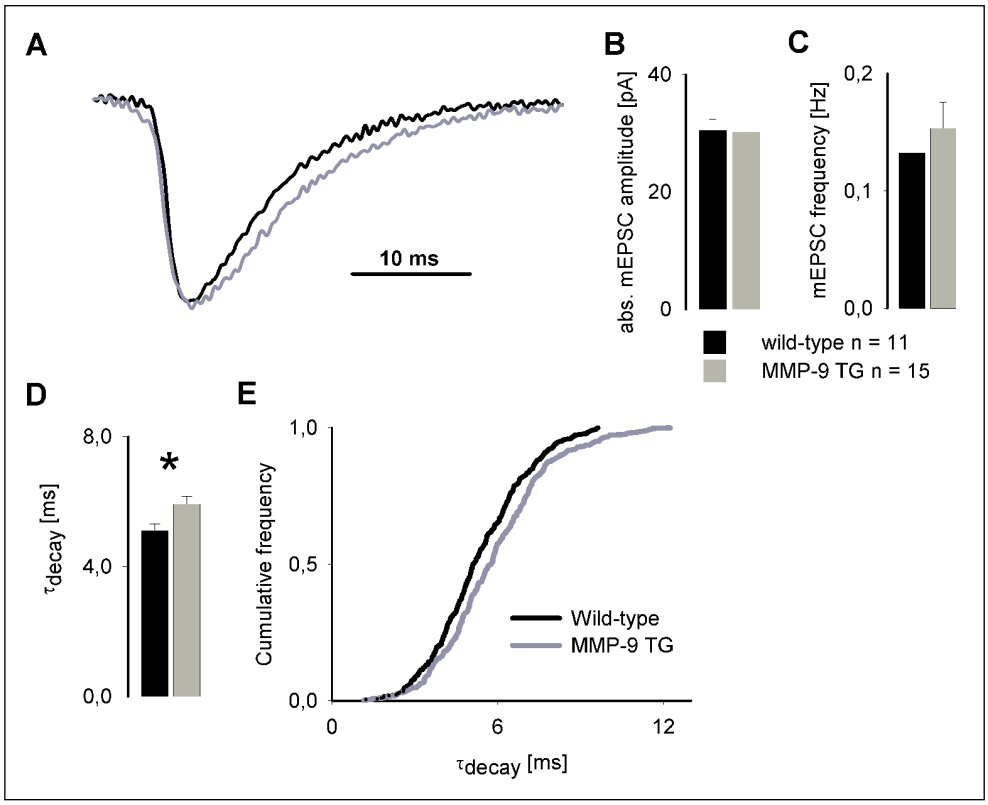

Fig. 3. MMP-9 overexpression is associated with increased mEPSCs decay time constant. (A) Example of normalized and superimposed mEPSCs recorded in CA1 region from wild-type (black) and from TG rats slices (grey). (B-D) Mean values of mEPSCs amplitudes (B), frequency (C) and decay time constant - τ_{decay} (D) recorded from WT and MMP-9 TG slices. Note statistically significant difference in decay kinetics between WT and MMP-9 TG groups (*t*-test). This difference is also evident in a cumulative distribution of mEPSCs decay time constants (E) for currents recorded from WT and TG slices (each cell is represented by an equal number of mEPSCs). * represents $P < 0.05$.

RESULTS

Overexpression of MMP-9 is associated with enhanced basal synaptic transmission

First we compared basal fEPSPs and fiber volleys in the CA1 region (*Fig. 1A*) in slices from rats overexpressing MMP-9 (TG rats) and their WT littermates. No difference in fiber volley amplitude was found, indicating similar susceptibility to excitation in TG and WT groups (two-way ANOVA, $f_{1,835} = 0.029$; $P = 0.84$; *Fig. 1B* and *1C*). Initial slope of fEPSPs evoked with increasing stimulation strengths (input-output relationship) showed larger values in slices from TG rats in comparison to WT controls (two-way ANOVA, $f_{1,835} = 77.6$; $P < 0.001$; *Fig. 1B* and *1D*). These results, presented as the slope of the fEPSP versus fiber volley amplitude, show that the overexpression of MMP-9 results in a significant increase in the basal fEPSPs (*Fig. 1E*). Thus, considering the lack of change in fiber volleys, a stronger synaptic transmission in TG group is implicated. Analysis of fEPSP amplitudes gave qualitatively the same results as fEPSP slope analysis (data not shown). The augmentation of synaptic transmission in MMP-9 overexpressing rats could result from changes in presynaptic neurotransmitter release, postsynaptic responsiveness or both. To examine presynaptic function, we analyzed paired-pulse facilitation ratio (PPR, 2nd to 1st fEPSP ratio, 50 ms interval). No change was observed in PPR between TG and WT rats (WT: 142 ± 5, n = 27; TG: 139 ± 4, n = 34; $P = 0.69$; *Fig. 1F*). Overall, these results suggest that larger fEPSPs in TG rats are attributable to a gain in postsynaptic responsiveness rather than to presynaptic mechanisms.

Overexpression of MMP-9 results in reduced late phase LTP in the CA3-CA1 projection

To elucidate the impact of massive MMP-9 overexpression on synaptic plasticity, long-term potentiation (LTP) was induced in slices from MMP-9 TG rats and in WT controls. Post-tetanic potentiation (PTP), a form of short-term plasticity, described here as normalized slope of the first response after high frequency stimulation (HFS), did not show any significant difference between the two genotypes (WT: 252 ± 24% of baseline slope, n = 10; TG: 268 ± 37%, n = 11, $P = 0.78$; *Fig. 2A*). In slices from WT animals, tetanization resulted in a marked late phase of LTP (mean fEPSP slope recorded between 90–100 min after HFS - 173 ± 17% of baseline values, n = 10). In MMP-9 overexpressing rats, LTP was significantly smaller (136 ± 21% of baseline slope, n = 11, $P < 0.05$; *Fig. 2A*, see also cumulative distribution *Fig. 2B*). Interestingly, a significant difference between the extents of LTP in WT and TG groups appeared 45 min after HFS, and remained different throughout the remaining recording period. This finding indicates that massive MMP-9 overexpression affects only the long-term plastic changes induced by titanic stimulation. Additionally, in WT group, HFS produced a pronounced upward shift in the I-O relationship (measured 90 min after HFS) reflecting prominent LTP maintenance ($f_{1,270} = 261$, $P<0.01$; *Fig. 2C*). Impairment of the LTP late phase in TG slices is manifested by only a slight shift in the I-O relationship with respect to that for WT group (for comparison of input-output curves 90 min after HFS between genotypes, $f_{1,255} = 52.7$, $P < 0.01$; *Fig. 2C*). Thus, MMP-9 overexpression in TG rats augments basic synaptic transmission in a CA3-CA1 projection, but impairs LTP maintenance.

Increased mEPSC decay time constant in slices from MMP-9 overexpressing rats

Alteration of basal synaptic transmission in CA3-CA1 synapses in TG rats suggests that excessive MMP-9 activity could influence glutamatergic synapses in this pathway. Since PPR values were not altered between TG and WT groups (*Fig. 1F*), we focused on the postsynaptic mechanisms and recorded, in the whole-cell mode (Vm = –70 mV), miniature EPSC (mEPSCs)

from CA1 pyramidal neurons in slices from TG and WT rats (*Fig. 3A*). We did not observe any significant differences in mEPSC amplitude (WT: 30.5 ± 1.9 pA, n = 11; TG: 30.2 ± 1.9 pA, n = 15; P = 0.98; unpaired Student's *t*-test; *Fig. 3B*) or frequency (WT: 0.13 ± 0.027 Hz, n = 11 cells; TG: 0.15 ± 0.022 Hz, n = 15 cells; P = 0.41; *Fig. 3C*). Considering that charge transfer mediated by a single mEPSCs critically depends on its time course, we additionally analyzed the decaying phase and rise time kinetics. We found that the decay time constant was significantly slower in TG in comparison to the WT group (WT: 5.1 ± 0.21 ms, n = 11; TG: 5.9 ± 0.25 ms, n = 15; P = 0.017; *Fig. 3A* and *3D*) but the onset kinetics did not show any difference (WT: 0.91 ± 0.074 ms, n = 8; TG: 1.09 ± 0.12 ms, n = 8; P = 0.31; not shown). Slower mEPSC decay kinetics in TG slices with respect to that in WT group is particularly clear in the cumulative distribution of the decay time constants (*Fig. 3E*).

DISCUSSION

In present study, we explored the hypothesis that MMP-9 overexpression in transgenic rats influences excitatory synaptic transmission and plasticity in the CA3-CA1 hippocampal pathway. It is worth emphasizing that the excess in MMP-9 activity is one of molecular mechanisms suggested to be responsible for e.g. synaptic impairments observed in fragile X syndrome (23-26). Therefore precise analysis of the detrimental effect of MMP-9 overexpression in hippocampus is of great importance. Presented data reinforces the notion that increased MMP-9 activity leads to changes in basal synaptic transmission and to impaired synaptic plasticity.

In our model, MMP-9 overexpression increased basal fEPSPs without affecting either the short-term plasticity or fiber volley amplitude indicating thus increased synaptic strength. This effect differs from our observation in mf-CA3 pathway, where basal fEPSPs were not affected by excess of MMP-9 (10) suggesting distinct mechanisms, but the nature of these differences remains unknown. Importantly, massive excess of MMP-9 in TG rats markedly impaired late-phase of LTP in the considered projection. However, it is worth noting that this effect appears weaker than that observed previously in mf-CA3 pathway (10), where impairment of LTP maintenance was nearly complete and emerged early after HFS. Moreover, it needs to be considered that overexpression of MMP-9 was associated with increased baseline fEPSPs in the CA3-CA1 pathway raising a possibility that increased synaptic strength in TG rats occurred in expense of reduced plasticity induced by tetanization. Taken together, the present data show that MMP-9 overexpression affects both baseline fEPSPs and synaptic plasticity in a qualitatively different manner than previously reported for mf-CA3 projection in the same model.

As an attempt to explore the source of differences between baseline fEPSPs in slices from TG and WT rats, mEPSCs were also recorded from pyramidal CA1 neurons and a slower decay in TG group was found. Rapid onset and decay kinetics clearly indicated non-NMDA identity of synaptic receptors (as expected for physiological magnesium level and strongly negative membrane potential). This is qualitatively similar to a recent finding that prolonged treatment of cultured neurons by recombinant autoactive MMP-9 caused a prolongation of mEPSC decaying phase (17). However, it is worth emphasizing that our present observation concerns slices, that is by far closer to physiological conditions than cell culture, and the source of MMP-9 is here endogenous. Nevertheless, the present study, together with previous findings (17), reinforces the notion that excessive MMP-9 action may give rise to a morphological and functional "juvenalization" of excitatory synapses. It remains to be established whether increased time duration of mEPSCs (with no effect on amplitude and frequency) in TG rats could contribute to enhanced basal fEPSPs in slices from the TG rats. In principle, considering that EPSPs mediated by non-NMDA receptors are short lasting and considering their time jitter in extensive neuronal network, prolonged EPSCs might favor enhancement in fEPSP amplitude, due to increased coincidence of these events. However, it seems unlikely that such a relatively minor effect on mEPSC duration is the only mechanism underlying our observations. Further studies, including e.g., single cell LTP or paired patch-clamp recordings will be needed to elucidate the mechanisms of these observations. In particular, it needs to be clarified how excessive MMP-9 affected the mEPSC decaying phase as a number of mechanisms might potentially be involved: switch in AMPA receptor subunit composition (27), post-translational modification of AMPA receptors or accompanying proteins (28), changes in lateral mobility or trapping of synaptic receptors (29). Additionally, dendritic spine changes induced with MMP-9 could shape the morphology of the synaptic cleft which could affect neurotransmitter diffusion and thereby postsynaptic responsiveness (30). Importantly, the lack of differences in mEPSC frequency and PPR ratios between TG and WT slices suggest that MMP-9 overexpression does not greatly interfere with presynaptic mechanisms pointing rather to the postsynaptic effect.

The present study underscores a crucial role of endogenous MMP-9 in shaping glutamatergic synaptic transmission and its plasticity in the classical CA3-CA1 hippocampal pathway. Noteworthy, the same strain of transgenic rats show increased susceptibility to epileptogenesis which seems consistent with reported here enhancement in basic synaptic responsiveness (18). The major unresolved problem is the molecular mechanism whereby excessive endogenous MMP-9 gives rise to observed effects. The main difficulty in resolving this issue is that a plethora of up- or downstream targets can be potentially involved including specific cleavage of synaptic proteins, e.g., pro-BDNF, β-dystroglycan, telencephalin, neuroligin-1 (12, 14, 31, 32). Additionally, active MMP-9 could affect synaptic AMPA or NMDA receptors. Indeed, recent studies revealed that exogenous MMP-9 activity caused acceleration of desensitization and deactivation kinetics and increased the lateral mobility of membrane NMDARs (33, 34). Moreover, induction of chemical LTP drives recruitment of GluA1- and GluA2-containing AMPARs into dendritic spines in MMP-dependent manner (14). In addition, soluble extracellular ICAM-5 domain, which is a product of MMP activity increases phosphorylation and dendritic insertion of GluA1-containing AMPARs (35).

It is noteworthy that, in contrast to the present results, transgenic mice with constitutive MMP-9 overexpression under the control of the platelet-derived-growth factor promoter showed increased late-phase LTP in CA3-CA1 projection *in vitro* and improvement in hippocampus-dependent learning (36). It needs to be emphasized that in the present study overexpression of auto-active MMP-9 form was achieved under synapsin I promoter. Other reasons for this apparent discrepancy could be the use different animal species (mice vs. rats) or distinct extent of MMP-9 overexpression resulting from the use of different promoters. Moreover, our rats overexpress auto-active form rather than pro-MMP-9 which has lower activation threshold in tissue (37).

In conclusion, we show that elevated MMP-9 expression in transgenic rats leads to potentiation of basal excitatory transmission and impairment of the maintenance phase of LTP in the CA3-CA1 projection. Interestingly, these effects are associated with a significant increase in the mEPSC decay time constant with respect to the WT group. These findings suggest marked alterations in functioning of considered neuronal

networks and raise a possibility that MMP-9 related alterations in basal glutamatergic transmission and its long-term plasticity may contribute to neurological and cognitive disorders.

Acknowledgements: Biological material used in the present study was kindly provided by prof. Leszek Kaczmarek from the Nencki Institute in Warsaw.

This work was supported by Polish Ministry for Science and Higher Education, Grant number: N N401 541540.

Conflict of interests: No declared.

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Received: September 2, 2014
Accepted: February 9, 2015

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