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ORIGINAL PAPER

Susceptibility to Aβo and TBOA of LTD and Extrasynaptic NMDAR-Dependent Tonic Current in the Aged Rat Hippocampus

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Abstract

Aging, as the major risk factor of Alzheimer's disease (AD), may increase susceptibility to neurodegenerative diseases through many gradual molecular and biochemical changes. Extracellular glutamate homeostasis and extrasynaptic glutamate *N*-methyl-*D*-aspartate receptors (NMDAR) are among early synaptic targets of oligomeric amyloid β (Aβo), one of the AD related synaptotoxic protein species. In this study, we asked for the effects of Aβo on long-term depression (LTD), a form of synaptic plasticity dependent on extrasynaptic NMDAR activation, and on a tonic current (TC) resulting from the activation of extrasynaptic NMDAR by ambient glutamate in hippocampal slices from young (3–6-month-old) and aged (24–28-month-old) Sprague–Dawley rats. Aβo significantly enhanced the magnitude of LTD and the amplitude of TC in aged slices compared to young ones. TBOA, a glutamate transporter inhibitor, also significantly increased LTD magnitude and TC amplitude in slices from aged rats, suggesting either an age-related weakness of the glutamate clearance system and/or a facilitated extrasynaptic NMDAR activation. From our present data, we hypothesize that senescence-related impairment of the extrasynaptic environment may be a vector of vulnerability of the aged hippocampus to neurodegenerative promotors such as Aβo.

Keywords Aging · Tonic current · Extracellular glutamate · Synaptic plasticity · NMDA receptors · Alzheimer

Introduction

A decline in cognition and memory occurs during aging even in the absence of neurodegenerative diseases [1, 2], due to gradual changes in the connectivity, structural organization and functional properties in central areas dedicated to learning and memory. One of the most prominent observations is the alterations in synaptic plasticity properties in the different hippocampal synaptic circuits (for review see [3, 4]). Changes in long-term potentiation (LTP), a cellular correlate of learning and memory, are consistently reported to occur during aging, but how long-term depression (LTD) expression is affected by age remains a matter of debate. LTD is implicated in different types of learning and memory and in cognitive flexibility, but more importantly, LTD may underlie synapse silencing and elimination in neurodegenerative diseases [5]. Whereas LTD induction appears to be facilitated in aged rodents (see [6]), other studies point to a weaker ability of hippocampal neuronal networks to express LTD [7–9]. These discrepancies may arise from the diversity of cellular induction mechanisms [5], that can be differentially affected by aging. Among those mechanisms, LTD can be induced by the activation of extrasynaptic NMDAR following glutamate transporters inhibition [10, 11], implicating extracellular glutamate level in synaptic depression. In the hippocampus, 90% of the extracellular glutamate uptake relies on astrocytes expressing the transporters GLAST and GLT-1 (see [12]). Transporters limit glutamate spill-out to extrasynaptic receptors and spillover to neighboring synapses [13]. Astrocytes are full partners of the synapse, but their neuroprotective properties are also challenged by senescence, playing critical roles in age-related neurological disorders [14]. If changes in

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glial functions, such as redox homeostasis and inflammatory response, are well known hallmarks of brain aging [15], astrocyte-dependent glutamate uptake has rarely been questioned. Some studies reported a lower uptake and a loss of glutamate transporter sites in the glutamatergic terminals of aged rodents [16–19]. In vivo voltammetric recordings have shown that the loss of GLAST transporters in the striatum of aged Fisher 344 rats was correlated with a slower clearance of glutamate [20]. In previous studies, we consistently described a decrease in GLAST and GLT-1 expression, and a significant reduction in the active glutamate uptake in the hippocampus from different strains of aged rat [21, 22].

Aging is the first risk factor of sporadic Alzheimer's disease (AD). AD is characterized by a progressive decline of cognitive and memory, and by a buildup of extracellular amyloid senile plaques and intra-neuronal neurofibrillary tangles. The accumulation of amyloid beta (Aβ) protein plays a major role in the pathogenesis of AD. The severity of dementia among AD patients better correlates with synaptic loss and oligomeric Aβ (Aβo) species than with plaque burden [23, 24]. Synapse loss is preceded by defects in synaptic transmission and plasticity, and it has been suggested that Aβo might be involved in the deregulation of these processes [25]. Experimentally, Aβo has been shown to inhibit LTP, and impairment of synaptic plasticity can be detected in vivo before the formation of insoluble Aβ deposits in AD transgenic mouse models (e.g., [26, 27]). Aβo originating from different sources has also been shown to facilitate the induction of LTD, when acutely applied to rat organotypic or ex vivo hippocampal slices [28], or injected in vivo into the hippocampus [29]. Interestingly, Aβo facilitates the induction of LTD by disrupting astrocyte-dependent glutamate uptake [30, 31] and increasing the activation of extrasynaptic NMDAR [32].

Rare studies have questioned the impact of Aβo in aged tissue. An in vivo experiment showed that Aβo injected into the dentate gyrus inhibited LTP in aged but not in young rats suggesting a change in susceptibility due to the underlying age-related neuroinflammation [33]. In the present study, we compared the impact of Aβo in young and aged hippocampal slices to determine if senescence can be a factor of susceptibility to this neuropathological promotor. We studied the effect of Aβo on two mechanisms dependent on both extrasynaptic NMDAR activation and extracellular glutamate level, (1) LTD and (2) a tonic current (TC) resulting from extrasynaptic NMDAR activation by ambient glutamate [34]. We observed that Aβo enhanced the magnitude of LTD in slices from both young and aged rats, but the facilitation of LTD was significantly higher in aged tissue. Aβo also significantly enhanced amplitude of the TC in aged slices compared to young ones. TBOA, a glutamate transporter inhibitor, reproduced the effects of Aβo on LTD and TC, and again the magnitude of effect was higher in aged slices. These data suggest that impairment of the extrasynaptic environment (such as an altered glutamate clearance and/or a facilitated extrasynaptic NMDAR activation), could be mechanisms of susceptibility of the aged hippocampal CA1 area to neurodegenerative promotors such as Aβo.

Materials and Methods

All animals were treated as approved by the University Paris-Descartes animal care and use committee and experiments were carried out in accordance with the guidelines outlined in the European Communities Council Directive (2010/63/EU). Animals were housed under standard conditions (12 h day/night rhythm, IVC racks, air-conditioned rooms, standard food, water ad libitum) at the approved animal core facility of INSERM U894 (Paris, France). Twenty-five young (3–6-month-old) and 25 aged (24–27-month-old) male Sprague–Dawley rats were used in this study, anesthetized by isoflurane before decapitation.

Conventional hippocampal slices were obtained as previously described [35]. Briefly, after removal from the skull, the brain was glued with cyanoacrylate adhesive to a metal block and submerged in the bath of a Leica 1200S vibroslicer. The artificial CSF bathing solution contained the following (in mM): 124 mM NaCl, 3.5 mM KCl, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26.2 mM, $NaHCO_3$, 1.2 mM, NaH_2PO_4, 11 mM, glucose and was maintained at 3 °C. Hippocampal slices were cut in the sagittal plane (400 µm thick) and stored in a recovering chamber in 95% O2, 5% CO2 bubbled aCSF at 28 °C. After a 1 h recovery period, all experiments were performed in bubbled aCSF at room temperature (21–24 °C).

Extracellular recordings were obtained from the CA1 stratum radiatum using glass micropipettes filled with NaCl 2M. Field excitatory postsynaptic potentials (fEPSPs) were evoked by electric stimulation of Schaffer collaterals/commissural pathway at 0.1 Hz with a bipolar tungsten stimulating electrode (20 µs duration). Basal synaptic transmission was estimated by averaging the slope of three successive fEPSPs using WinLTP software. The slope was measured between two cursors, one placed as soon after the fiber volley as the fEPSP slope becomes linear, and the second one before it begins to asymptote toward the peak. LTD of synaptic transmission was evaluated by a 2 Hz low frequency stimulation (LFS) paradigm for 10 min. Responses were recorded for at least 45 min

after LFS. Drugs were applied in the perfusion bath for at least 15 min before LFS. Experiments under control conditions were systematically performed on the same day.

Whole-cell patch-clamp recordings of CA1 pyramidal neurons were obtained at room temperature, with borosilicate patch pipettes (open-tip resistance 5 MΩ) filled with a solution containing 140 mM $CsCH_4O_3S$, 6 mM CsCl, 2 mM $MgCl_2$, 10 mM HEPES, 1.1 mM EGTA, 5 mM QX-314, and 4 mM ATP, (pH 7.3; 290–310 mosm). Voltage-clamp recordings were performed with an Axo-Patch 1-D amplifier (Axon Instruments, Union City, CA). Signals were filtered at 2 Hz and acquired at a sample rate of 20 kHZ using a National Instruments digitizer and WinLTP software [36]. Access resistance and capacitance were compensated online. Access resistance typically was 10–20 MΩ and remained relatively stable during experiments.

Experiments assessing tonic NMDAR current were done in the presence of the $GABA_A$ receptor ($GABA_AR$) blocker bicuculline methiodide (10 µM; Abcam), the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) blocker (NBQX; 10 µM; Ascent Scientific), and the voltage-gated Na^+ channel blocker tetrodotoxin (TTX; 1 µM; Abcam). *D*-2-Amino-5-phosphopentanoic acid (AP-5; 50 µM; Ascent Scientific) was bath applied to evaluate the NMDA component of the holding current to maintain the recorded neuron at a + 40 mV potential. The amplitude of the TC was measured as the difference in the mean holding current values before and after application of AP-5. The change in baseline noise produced by AP-5 was also assessed. Peak-to-peak amplitude of the baseline noise was measured with Spike 2 software before and after effects of the drugs. Miniature excitatory postsynaptic currents (mEPSCs) were recorded at a holding potential of − 60 mV. TTX (1 µM) and bicuculline methiodide (10 µM) were added to the bath to block action potentials and $GABA_A$R-mediated inhibitory postsynaptic currents, respectively. Miniature EPSCs were analyzed using the Spike2 program (CED, Cambridge, UK). Peak events were automatically detected using an amplitude threshold of 1.5 times the peak-to-peak baseline noise. Frequency of mEPSCs occurrence was estimated during at least 2 min recording at − 60 mV.

Other drugs used in this study include human recombinant Aβ 1–42 (200 nM, Bachem, Weil and Rhein, Germany), and the glutamate transporter inhibitor TBOA (*D,L*-threo-beta-benzyloxyaspartic acid, Tocris, Illkirch, France). Oligomers of amyloid beta protein were prepared as previously described ([32] adapted from [37]). Recombinant Aβ 1–42 peptide was dissolved at 1 mM in 1,1,1,3,3,3-hexafluoro-2-propanolol (HFIP, Sigma, St-Louis, USA) and aliquoted. The HFIP was allowed to evaporate in a fume hood and resulting peptide films were store at − 80 °C. For oligomerization of the peptide, HFIP-treated aliquots were carefully and completely resuspended in 5 µl dimethylsulfoxide (DMSO) by pipette mixing, and addition of 108 µl of aCSF with immediate vortexing. The mixture was then incubated at 4 °C for 24 h on a stirring machine. All drugs were diluted directly in aCSF from stock solutions prepared in distilled water or in DMSO.

Statistical Analysis

Two-tailed *t*-tests and paired *t*-tests were used for group comparisons in most cases. Values are mean ± SE. Unless otherwise stated, n was the number of recorded cells in patch-clamp protocols, and slices in extracellular experiments. Statistical tests were performed using StatView Software (SAS Scientific Computing, USA). For analysis of synaptic plasticity, *p* values were calculated using multivariate analysis of variance followed by Tukey's post hoc tests (StatView software) to account for the correlations inherent to repeated measures in electrophysiological recordings.

Results

LTD

Effect of Aβo on LTD

The impact of Aβo on LTD was first tested. We applied synthetic Aβo at 200 nM, a concentration which is in the pathological range of the hormetic dose–response curve [38], and that we have previously shown to inhibit LTP in the CA1 hippocampus of adult mice [32]. Aβo, perfused for 20 min, comparatively depressed fEPSPs slope during baseline in young and aged slices (84.6 ± 2.9% of the baseline in young, n = 17 slices from 12 rats, vs 84.2 ± 3.2% in aged, n = 15 slices from 12 rats, ns). We first demonstrated that this chemical LTD (cLTD) was stable along time by applying Aβo for 70 min in aged slices (n = 7, data not shown). cLTD induced by Aβo shares common mechanisms with electrical-induced LTD (eLTD) (see [39] for references), and eLTD could be modified by cLTD-induced metaplastic mechanisms. Consequently, we compared the total LTD (cLTD + eLTD) value between young and aged slices. We showed that total LTD in young animals reached 78.6 ± 1.7% in control versus 62.3 ± 9.8% in Aβo (*$p = 0.04$) (Fig. 1A). The effect of Aβo was stronger in aged tissues, reaching 88.5 ± 2.2% in control versus 49.5 ± 9.6% in Aβo. This difference was highly significant (***$p = 0.0001$) (Fig. 1B).

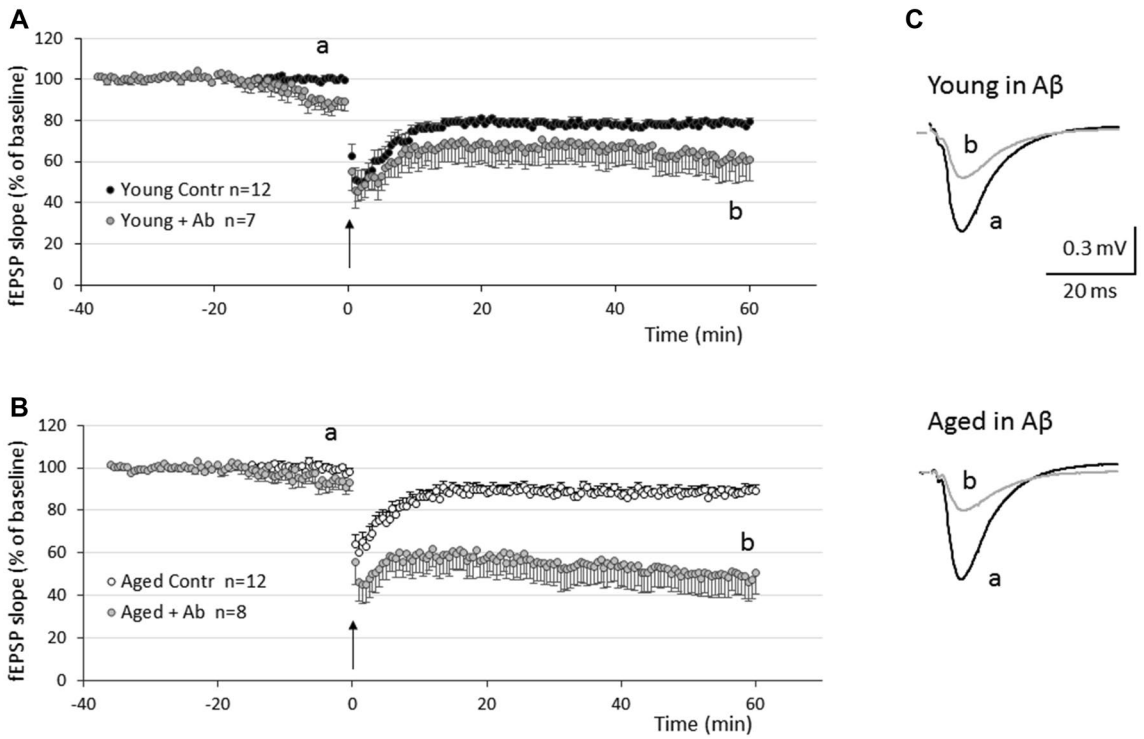

Fig. 1 Effect of Aβo on time course of LTD in slices from young (**A**) and aged (**B**) rats. **A**—effect of Aβo on LTD in young rats. LTD is expressed as a percent change in fEPSP slope across time and is induced by a LFS (2 Hz, 10 min) applied at t0 (arrow). Black circles represent mean LTD recorded in control slices from young rats. Grey circles represent the mean LTD recorded in slices from young rats in the presence of Aβo (200 nM), applied in the superfusing medium 15 min before t0 and maintained throughout the experiment. Aβo in young rats has no significant effect on LTD. **B**—same experiments in aged rats. Mean LTD in aged control rats is represented in white circles. Superimposed in grey circles is the mean LTD recorded in slices from aged rats in the presence of Aβo (200 nM), applied in the superfusing medium 15 min before t0 and continuously applied throughout the experiment. Difference between aged control and aged + Aβo was highly significant (***$p < 0.0001$). **C**—examples of individual fEPSPs recorded in young and aged slices in Aβ at time points a and b indicated in A and B

Effect of TBOA on LTD

We next acutely challenged glutamate uptake by applying the glutamate transporter blocker TBOA. TBOA also induce cLTD of fEPSPs through activation of extrasynaptic NMDAR receptors [10]. When applied at 20 µM, TBOA induced a significant depression of the fEPSPs slope in hippocampal slices from aged rats (81.2 ± 3.8% of the baseline, n = 10 slices from 10 aged rats), compared to those of young rats (94.0 ± 3.8%, n = 8 slices from eight young rats, *$p = 0.04$; Fig. 2). A control set of experiments was performed to check for the stabilization along time of the TBOA-induced cLTD. Stabilization 77.5 ± 7.5% (n = 7 slices in two aged rats) was achieved in 20 min, and remained unchanged along a recording period of 70 min (data not shown). Electrically-evoked LFS was then applied after 20 min of TBOA perfusion to induce eLTD. We showed that total LTD (cLTD + eLTD) in young animals reached 78.6 ± 1.7%, n = 12 in control versus 56.2 ± 6.4%, n = 7 in TBOA (***$p = 0.0007$) (Fig. 2A). The effect of TBOA was stronger in aged tissues reaching 88.5 ± 2.2%, n = 12, versus 40.5 ± 4.7%, n = 8 (***$p < 0.0001$) (Fig. 2B).

Tonic Current

The aged hippocampus seems more susceptible to synaptic depression when acutely faced to pathological Aβo or elevated extracellular glutamate level. We therefore envisaged the age-related alteration in extracellular glutamate homeostasis as a pre-existing vulnerable mechanism in aged tissue. Searching for a more direct evaluation of this alteration, we recorded a TC in CA1 pyramidal neurons, mediated by the activation of NMDAR by ambient glutamate. NMDARs are located at both synaptic and extrasynaptic sites, and their activation produces phasic NMDAR current through fast vesicular release and TC through action of ambient glutamate, respectively. The tonic activation of NMDAR by ambient glutamate was assessed in whole-cell patch-clamped CA1 pyramidal neurons from young to aged rats, and expressed as a

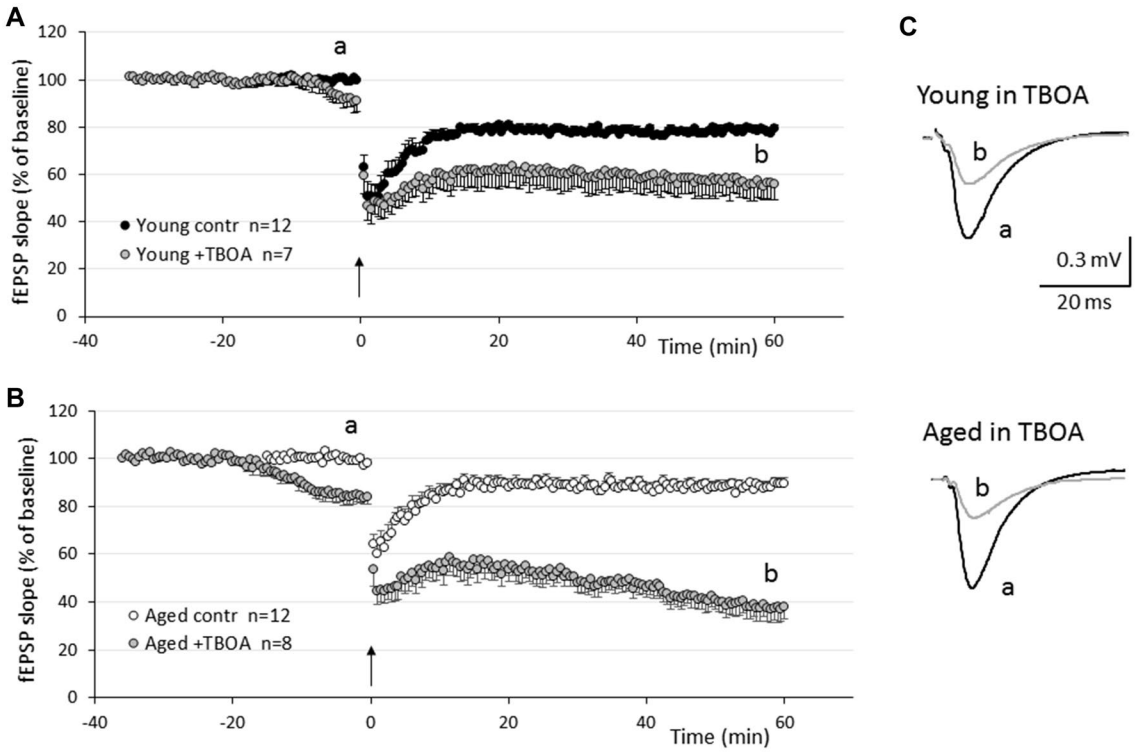

Fig. 2 Effect of TBOA on time course of LTD in slices from young (**A**) and aged (**B**) rats. LTD is expressed as a percent change in fEPSP slope along time **A**—LTD was applied on slices from young rats recorded in control conditions (black squares) or in the presence of TBOA (20 µM, grey squares). LTD was induced by a LFS (2 Hz, 10 min) applied at t0 (arrow). TBOA (20 µM) was applied in the superfusing medium after a 15 min baseline. Recording was interrupted during LFS so this period of time does not appear on the figure. LFS induces a depression of the fEPSP slope which recovers partially to reach a stable level of depression about 20 min after stimulation. The level of LTD was stronger in the presence of TBOA (**$p<0.01$). TBOA was applied throughout the experience. **B**—same experiments in slices from aged rats. We observed a stronger effect of TBOA in aged compared to young slices (***$p=0.0002$). The depression induced by TBOA on fEPSP before LFS was taken into account for the calculation of LTD. **C**—superimposed examples of individual fEPSPs recorded in young and aged slices in the presence of TBOA at time points a and b indicated in A and B

negative shift in the holding current following NMDAR blockade by AP-5 (50 µM) from a holding potential of +40 mV. This holding potential allowed for the complete removal of the voltage dependent blockade of NMDARs by external Mg^{2+} and their possible activation by ambient glutamate. Experiments were performed in the presence of AMPAR, $GABA_AR$, and voltage-gated Na^+ channel blockers (Fig. 3A). The TC amplitude recorded in control conditions was significantly enhanced in neurons from aged rats (205.5 ± 11.9 pA, n = 33 cells in 25 rats) when compared to neurons from young rats (118.3 ± 7.0 pA, n = 35 cells in 25 rats, ***$p<0.001$) (Fig. 3B).

The contribution of spontaneous action potential (sAP)-independent release of glutamate can affect measurements of tonic NMDAR current by increasing ambient glutamate concentration, and could participate in the enhancement of TC amplitude in aged rats. The sAP-independent release of glutamate was therefore evaluated in both populations by recording AMPAR-dependent miniature excitatory postsynaptic currents (mEPSCs) at a holding potential of − 60 mV. The frequency of mEPSCs occurrence was comparable between young and aged neurons (0.85 ± 0.06 Hz in young, n = 27 cells in 20 rats, and 1.0 ± 0.06 Hz in aged neurons, n = 26 cells in 23 rats, $p=0.17$), as well as the amplitude of the miniature events (18.6 ± 1.3 pA in young, n = 27 cells in 20 rats, and 20.7 ± 1.5 pA in aged neurons, n = 26 cells in 23 rats, $p=0.44$), suggesting a comparable sAP-independent vesicular release in young and aged rats (Fig. 4A, B).

Tonic NMDAR current was also assessed as the difference in baseline noise before and after NMDAR antagonism. Holding neurons at +40 mV generates an increase in baseline noise amplitude, reflecting the introduction of NMDAR mEPSCs to the baseline current resulting from sAP-independent release (Fig. 5A). As shown in Fig. 5B, peak-to-peak baseline noise amplitude at +40 mV was comparable in young and aged rats (52.7 ± 3.8 pA and 58.5 ± 2.7 pA respectively, $p=0.22$) before AP-5, and reduced by the drug to

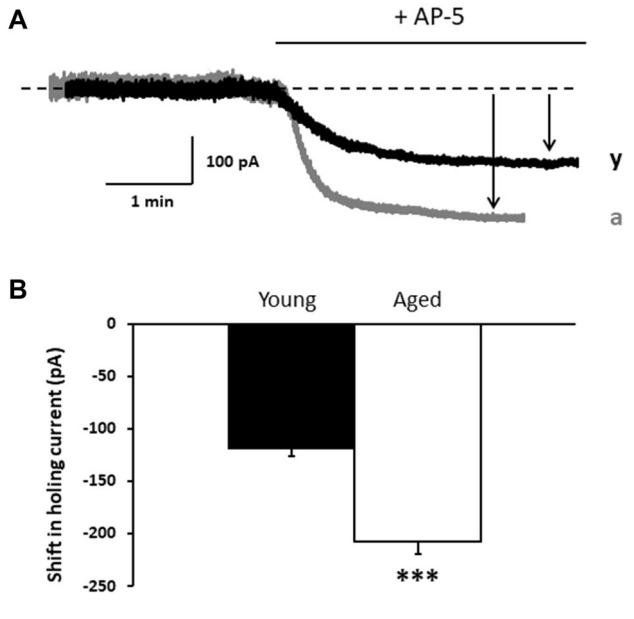

Fig. 3 Tonic NMDAR dependent current in CA1 pyramidal neurons from young to aged rats. **A**—Examples of the negative shift of the holding current as a result of 50 µM AP-5 application from a holding potential of + 40 mV (dashed line) are shown in a CA1 neuron from a young (black trace), and aged rat (grey trace). Arrows indicate difference in the shift amplitude relative to baseline. **B**—Summary of tonic NMDAR-dependent current expressed as a shift in holding current during AP-5 application (50 µM), recorded in young (black, n = 35) and aged (white, n = 33) CA1 neurons at a + 40 mV holding potential. The AP-5 induced shift was significantly enhanced in neurons from aged rats (***$p < 0.001$)

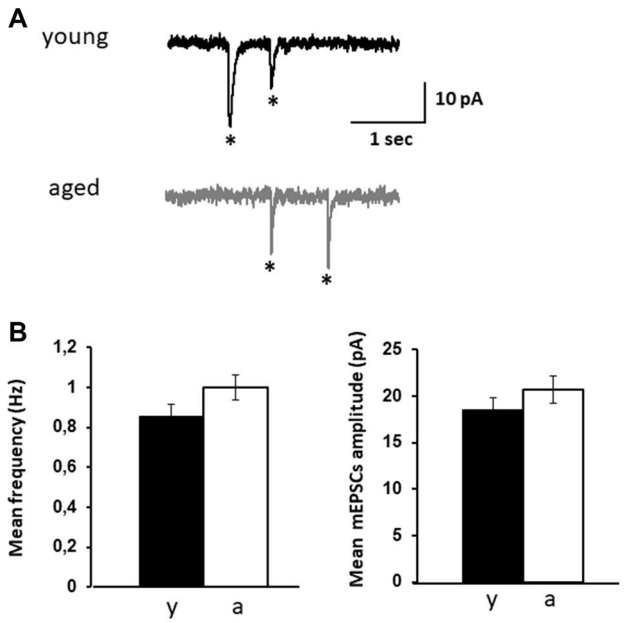

Fig. 4 sAP independent release of glutamate in young and aged CA1 pyramidal neurons. **A**—Examples of miniature excitatory post-synaptic currents (mEPSCs) in CA1 pyramidal cells in slices from young to aged rats. mEPSCs are marked by asterisks. Holding potential was − 60 mV. **B**—Pooled mEPSCs frequency and amplitude in young (n = 27; black box) and aged (n = 26; white box) neurons

26.9 ± 2.7 pA in neurons from young (n = 23 cells in 19 rats) and 28.2 ± 1.9 pA in neurons from aged rats (n = 22 cells in 20 rats). When expressed as percent of control, reduction of the baseline noise by AP-5 was comparable in both populations (51.7 ± 3.5% in young vs 48.7 ± 2.6% in aged neurons, $p = 0.49$, Fig. 5B). This result, together with the unaffected frequency of glutamate release estimated at − 60 mV, is in favor of a comparable contribution of NMDAR mEPSCs input to the TC in young and aged rats. We therefore hypothesize that the difference in TC amplitude between young and aged rats may arise mainly from extrasynaptic changes.

TBOA was then applied at 100 µM in slices from young to aged rats to inhibit glutamate transporters. TBOA rapidly and reversibly increased the TC amplitude (Fig. 6A) in both young and aged rats, as expressed by the positive shift of the holding current at + 40 mV. This increase was significantly higher in aged (508.9 ± 58.3 pA, n = 9 cells in 7 rats) than in young neurons (232.7 ± 39.8 pA, n = 11 cells in 10 rats, ***$p < 0.001$, Fig. 6B, C). TBOA also increased the amplitude of the peak-to-peak noise compared to baseline (see Fig. 5A). This increase was similar in young (77.9 ± 9.9 pA, n = 11 in 10 rats) and aged neurons (83.5 ± 6.3 pA, n = 9 in 7 rats, $p = 0.64$). These data indicate that the additional NMDA-dependent mEPSCs generated by TBOA was of comparable contribution to the TC in aged and young neurons, and could not account for the difference in TC amplitude.

Aβo effect was next tested on TC amplitude. When applied at 200 nM, Aβo increased the TC amplitude (Fig. 7A) in both young and aged rats, as expressed by the positive shift of the holding current at + 40 mV. The increase was significantly higher in aged (244.0 ± 24.2 pA, n = 5 cells in 5 rats) than in young neurons (96.0 ± 22.4 pA, n = 5 cells in 5 rats, **$p < 0.01$, Fig. 7B). Aβo did not modify the peak-to peak noise (Fig. 7C). Peak-to peak noise quantification was similar in young (50.4 ± 10.9 pA in control and 68.8 ± 13.5 pA in the presence of Aβo, n = 5 cells in 5 rats, $p = 0.32$) and aged neurons (53.6 ± 10 pA in control and 69.2 ± 13.6 pA in the presence of Aβo, n = 5 cells in 5 rats, $p = 0.47$), and not significantly different from control baseline noise. This latter result suggests a dominant impact of Aβo on the extrasynaptic component of the TC.

Discussion

Our present data describe a facilitation effect of acutely applied Aβo on LFS-induced LTD in aged rat hippocampal slices. The facilitation of LTD by exogenous Aβo has

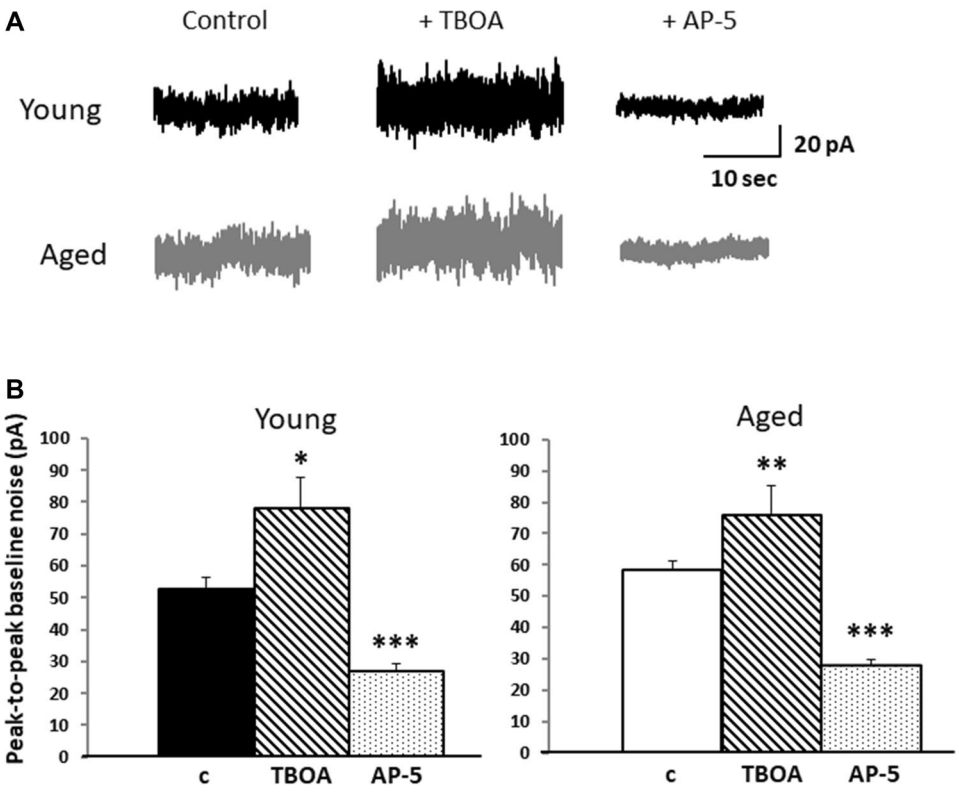

Fig. 5 Amplitude of the holding current (hc) noise at +40 mV in young and aged CA1 pyramidal neurons. **A**—Hc noise in baseline conditions, in the presence of TBOA (100 µM) and AP-5 (50 µM) in CA1 pyramidal neurons from young (black traces) to aged (grey traces) rats. **B**—Pooled data of TBOA and AP-5 effect on hc current noise amplitude recorded in neurons from young (left) to aged rats (right). TBOA statistically increased peak-to-peak noise in young (*$p=0.03$) and in aged (**$p=0.004$) rats, but no statistical difference was observed between young and aged rats ($p=0.64$)

been widely studied in young animals [30, 31, 40], and implicate NMDAR and metabotropic glutamate receptor (mGluRs) activation. Synthetic Aβo peptides have been reported to facilitate LTD induction in an NMDAR dependent manner in vivo [29], and extracted buffer soluble Aβo from AD patients brain facilitated LTD induction in the CA1 region of mouse hippocampus by an mGluR-dependent mechanism [28]. Production of Aβo in organotypic slices obtained from APP-overexpressing mice decreased AMPAR at the cell surface by a mechanism partially occluding LTD [41]. However, all these effects of Aβo have been demonstrated in young AD mice model tissue or following acute application in young brain tissue, therefore shunting the importance of senescence, the major factor of susceptibility to AD. Aβo has been shown to share some common mechanisms of action with TBOA in adult animals [30], i.e. facilitating LTD by disrupting astrocytic glutamate uptake, and activating extrasynaptic GluN2B-containing NMDAR [31, 42]. In our present experiments, TBOA significantly facilitated LFS-induced LTD in aged slices when compared to young ones, suggesting an extrasynaptic dysregulation in the senescent hippocampus. We then recorded an extrasynaptic NMDAR-dependent TC induced by ambient glutamate in CA1 pyramidal cells, and found that TBOA and Aβo significantly enhanced TC amplitude in aged slices. Further experiments need to be performed to understand how Aβo can affect glutamate uptake and extrasynaptic NMDAR and to what extent in the senescent context. Moreover, Aβo may also act on multiple sites on the GABAergic and cholinergic systems [43, 44] that may indirectly lead to glutamate impairments [45].

Interestingly, we observed a significant increase of the control TC amplitude in aged slices, suggesting an age-associated alteration of the extrasynaptic compartment, which could either arise from an impaired glutamate clearance and/or from a facilitated extrasynaptic NMDAR activation. According to Le Meur et al. [34], the tonic extrasynaptic NMDAR-dependent current in the hippocampus is dependent on glutamate predominantly originating from a non-vesicular origin. This conclusion raised from the inhibition of glutamine synthetase (GS), the astrocytic enzyme responsible for conversion of glutamate to glutamine, which caused an increase in tonic NMDAR current in hippocampal pyramidal neurons. Based on this observation, Le Meur et al. suggested that ambient glutamate was mostly of glial origin. In our experiments, we verified that the spontaneous release of glutamate was unchanged in slices from aged rats, which excluded a higher contribution of the vesicular release to the aged TC. Complementary

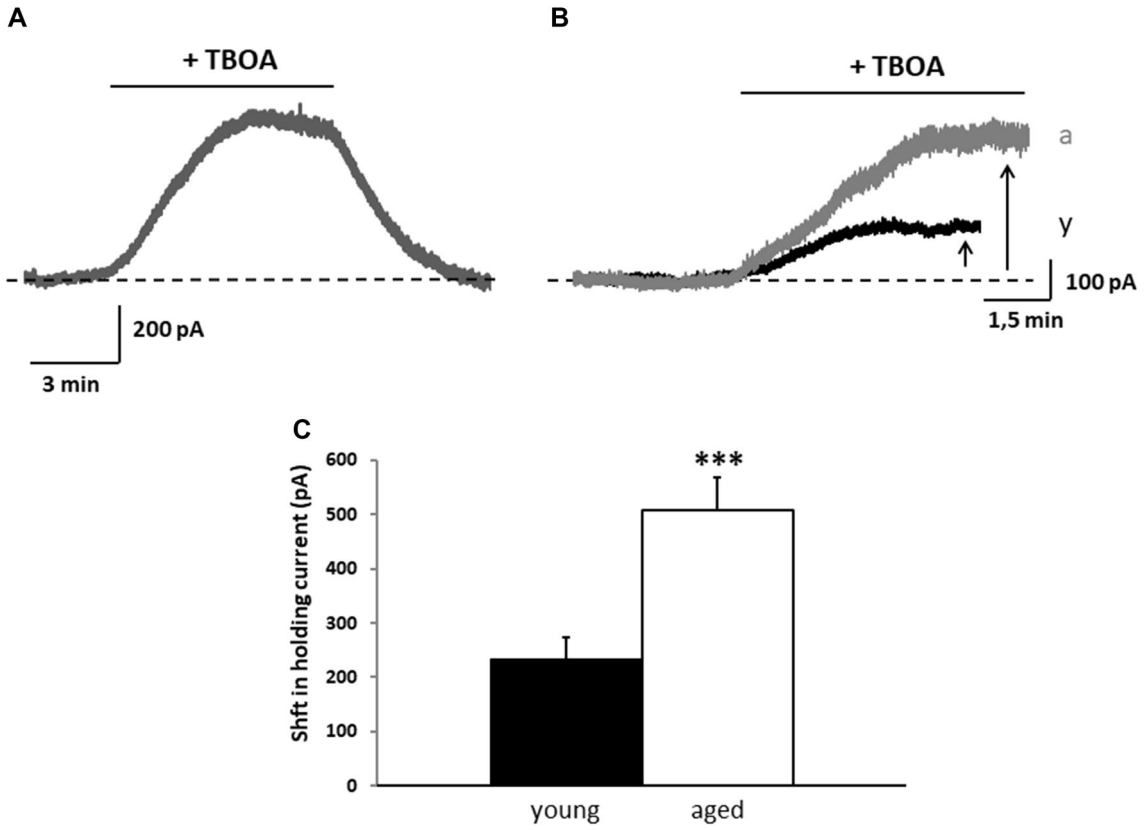

Fig. 6 Effect of TBOA on the TC in CA1 pyramidal neurons from young to aged rats. **A**—Representative trace of the increase in TC amplitude induced by TBOA. The hc rapidly returned to baseline upon TBOA washed out. **B**—Typical examples of the positive shift in the holding current as a result of 100 µM TBOA application from a holding potential of +40 mV (dashed line) are shown in a CA1 neuron from a young (black trace), and aged rat (grey trace). Arrows indicate difference in the shift amplitude relative to baseline. **C**—Positive shift in holding current induced by TBOA (100 µM) application, recorded in young (black, n = 11) and aged (white, n = 8) CA1 neurons at a + 40 mV holding potential. The effect of TBOA on the TC was significantly stronger in neurons from aged rats (***$p < 0.001$)

experiments need to be performed in the presence of bafilomycin (as performed in [34, 46]), to suppress vesicular glutamate release and to fully isolate the extrasynaptic component of the TC, but we may assume from our present data that the increase in TC amplitude observed in aged rats was more largely due to extracellular glutamate from non-synaptic origin.

During aging, glial cells and particularly astrocytes, are subjected to reactive gliosis [47, 48] (see [14] for a review), characterized by a build-up in glial fibrillary acid protein (GFAP). In a previous study [21], we have described an increase in GFAP in the hippocampal CA1 area of aged rats, using western-blot analysis. With the same method, we did not observe any change in GS protein content; however this does not exclude a decrease in enzyme activity that could account for the change in TC amplitude in aged rats. Reactive gliosis affects volume neurotransmission during physiological and pathological states and a reduction in the extracellular space during senescence influences glutamate spill-over [49, 50]. Moreover, we and others [16, 19–22] reported a reduction in active glutamate uptake, a decrease in astrocyte glutamate transporters expression, and a slower glutamate uptake in aged rats. These different age-related modifications of astrocytes may have impacted the extracellular repartition of glutamate and the tonic activation of extrasynaptic NMDAR, which may ultimately account for an enhancement of the tonic current.

In conclusion, our present data suggest that impairments in the extrasynaptic environment during senescence may contribute to alterations in synaptic plasticity and to an increased susceptibility to neuropathological promotors such as Aβo.

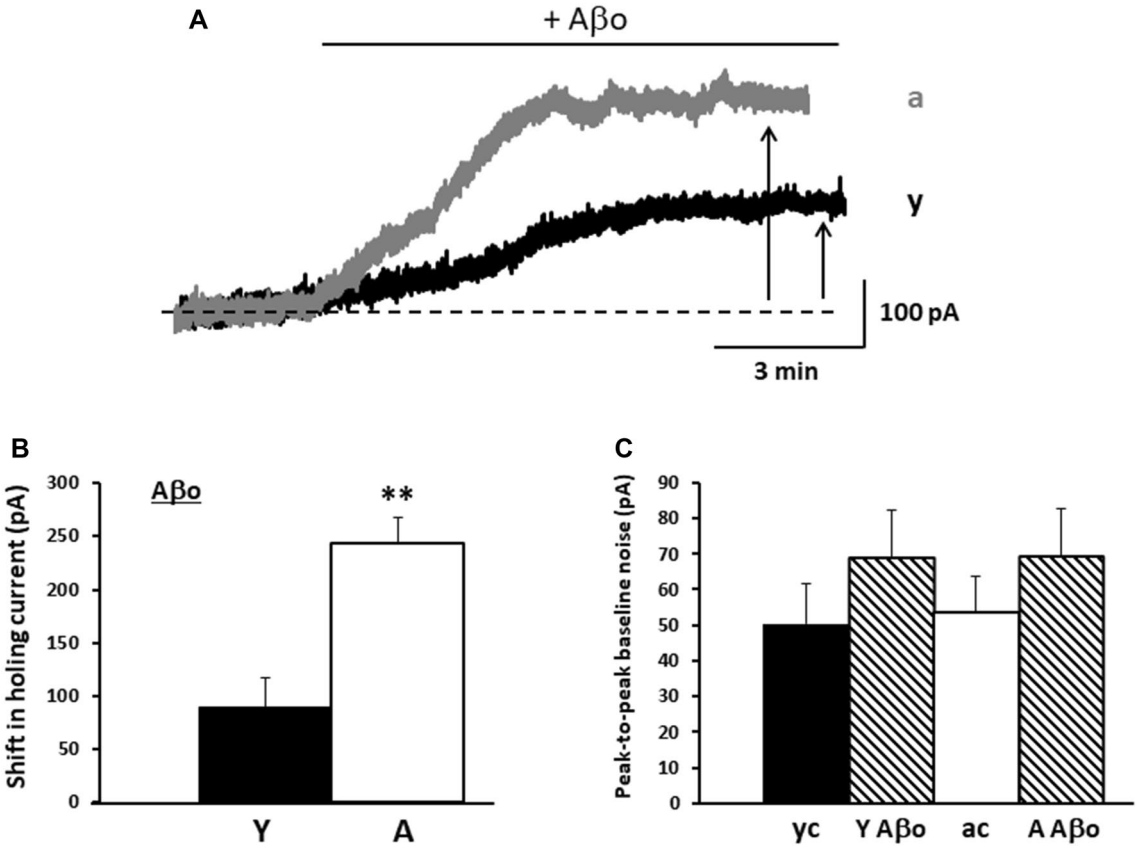

Fig. 7 Effect of Aβo on the TC in CA1 pyramidal neurons from young to aged rats. **A**—Positive shift in the holding current as a result of 200 nM Aβo application from a holding potential of +40 mV (dashed line) in a CA1 neuron from a young (black trace), and aged rat (grey trace). Arrows indicate difference in the shift amplitude relative to baseline. **B**—Quantification of the shift in holding current induced by Aβo (200 nM) application, recorded in young (black, n = 5) and aged (white, n = 5) CA1 neurons at a + 40 mV holding potential (**$p < 0.01$). **C**—Aβo has no effect on the peak-to-peak noise either in young or aged rats

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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