Circuit-specific changes in D-serine-dependent activation of the *N*-methyl-D-aspartate receptor in the aging hippocampus

M. Labarrière · F. Thomas · P. Dutar · L. Pollegioni ·
H. Wolosker · J.-M. Billard

Received: 11 February 2014 / Accepted: 27 July 2014 / Published online: 20 August 2014
© American Aging Association 2014

Abstract Age-related memory deficits have recently been associated with the impaired expression of D-serine-dependent synaptic plasticity in neuronal networks of the hippocampal CA1 area. However, whether such functional alterations are common to the entire hippocampus during aging remains unknown. Here, we found that D-serine was also required for the induction of *N*-methyl-D-aspartate receptor (NMDA-R)-dependent long-term potentiation (LTP) at perforant path-granule cell synapses of the dentate gyrus. LTP as well as isolated NMDA-R synaptic potentials were impaired

in slices from aged rats, but in contrast to the CA1, this defect was not reversed by exogenous D-serine. The lower activation of the glycine-binding site by the endogenous co-agonist does not therefore appear to be a critical mechanism underlying age-related deficits in NMDA-R activation in the dentate gyrus. Instead, our data highlight the role of changes in presynaptic inputs as illustrated by the weaker responsiveness of afferent glutamatergic fibers, as well as changes in postsynaptic NMDA-R density. Thus, our study indicates that although NMDA-R-dependent mechanisms driving synaptic plasticity are quite similar between hippocampal circuits, they show regional differences in their susceptibility to aging, which could hamper the development of effective therapeutic strategies aimed at reducing cognitive aging.

Electronic supplementary material The online version of this article (doi:10.1007/s11357-014-9698-0) contains supplementary material, which is available to authorized users.

M. Labarrière · F. Thomas · P. Dutar · J.-M. Billard (✉)
Centre de Psychiatrie et Neurosciences, Université Paris
Descartes, Sorbonne Paris Cité,
UMR 894, Paris 75014, France
e-mail: jean-marie.billard@inserm.fr

L. Pollegioni
Dipartimento di Biotecnologie e Scienze della Vita, Università
degli studi dell'Insubria,
via J.H. Dunant 3, 21100 Varese, Italy

L. Pollegioni
The Protein Factory, Centro Interuniversitario di
Biotecnologie Proteiche, Politecnico di Milano, ICRM CNR
Milano, and Università degli Studi dell'Insubria,
via Mancinelli 7, 20137 Milano, Italy

H. Wolosker
Department of Biochemistry, Rappaport Faculty of Medicine,
Technion-Israel Institute of Technology,
Haifa 31036, Israel

Keywords Dentate gyrus · Synaptic plasticity · LTP ·
NMDA receptors · Glycine-binding site · Learning and
memory

Abbreviations

aCSF	Artificial cerebrospinal fluid
DAAO	D-amino acid oxidase
fEPSP	Field excitatory postsynaptic potential
D-APV	D-2-amino-5-phosphonovalerate
DsdA	D-serine deaminase
I _{ES}	Index of synaptic efficacy
KO	Knockout
LTP	Long-term potentiation

NBQX	2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide
NMDA-R	<i>N</i> -methyl-D-aspartate receptor
PFV	Presynaptic fiber volley
SR	Serine racemase

Introduction

The decline in memory abilities commonly encountered during normal aging, especially with regard to newly acquired information, significantly alters the quality of life of the elderly (Luo and Craik 2008). Because its integrity is critical for normal memory function, the hippocampal formation has been the focus of much experimental interest, and many structural and functional changes have been characterized throughout the aging hippocampus. Among the different mechanisms proposed to explain cognitive aging are deficits in synaptic plasticity such as those in the long-term potentiation (LTP) of glutamate transmission (see Barnes 2003; Billard 2006; Burke and Barnes 2010; Foster 2006; Lynch 2010; Rosenzweig and Barnes 2003 for reviews), a property of neuronal networks thought to trigger memory formation (Izquierdo 1991; Kim and Linden 2007; Lisman and McIntyre 2001; Lynch 2004; Martin et al. 2000). Although experimental evidence shows that LTP is impaired in the CA1 area (Barnes et al. 1996; Clayton et al. 2002; Foster 2002; Moore et al. 1993; Shankar et al. 1998; Turpin et al. 2011) as well as in the dentate gyrus of aged rodents (Barnes and McNaughton 1985; Barnes et al. 2000; Griffin et al. 2006; Lynch 1998), whether the underlying mechanisms in these different hippocampal regions share common features or are specific to each area is not well understood (Barnes 1994). This is a moot point considering that age-related changes in the number of synaptic contacts have been found in the dentate gyrus and CA3 but not in the CA1 (see Burke and Barnes 2010 for review), suggesting the differential susceptibility of glutamatergic circuits within the hippocampus to aging processes.

The activation of the *N*-methyl-D-aspartate (NMDA-R) subtype of glutamate receptors is a critical step for LTP (Collingridge and Bliss 1995; Malenka and Nicoll 1993). The amino acid D-serine, by binding to the NMDA-R glycine-binding site, regulates the expression

of functional plasticity in numerous brain areas including the hippocampal CA1 (reviewed in Billard 2012). Importantly, D-serine levels are reduced in the aging hippocampus due to the weaker expression of its synthesizing enzyme, serine racemase (Potier et al. 2010). In the CA1 area, this decrease impairs NMDA-R activation and LTP expression, indicating the critical role of D-serine in the mechanisms underlying hippocampus-related cognitive aging (Haxaire et al. 2012; Mothet et al. 2006; Turpin et al. 2011). In the dentate gyrus also, NMDA-R activation and LTP expression are affected by age (Barnes et al. 2000; Diana et al. 1994; Dieguez and Barea-Rodriguez 2004; Sierra-Mercado et al. 2008), but whether D-serine is involved in these dysfunctions remains unknown. Determining if the D-amino acid significantly contributes to the age-related deterioration of functional plasticity in the different hippocampal circuits is an important issue in the search for therapeutic strategies targeting cognitive aging.

In light of the critical role of D-serine in the functional plasticity of CA1 circuits throughout life span, we therefore asked whether the D-amino acid also drives age-related changes at perforant pathway-granular cell synapses of the dentate gyrus.

Methods

All experiments were carried out in accordance with the European Communities Council Directive (86/809/EEC) regarding the care and use of animals for experimental procedures. The experiments were conducted with 4- to 6-month-old “adult” ($n=45$) and 23- to 26-month-old “aged” ($n=15$) male Sprague-Dawley rats purchased from Charles River (France). Rats were housed by threes in Plexiglas cages and maintained on a controlled light-dark cycle at constant temperature (22 ± 2 °C), with ad libitum access to food and water.

Electrophysiology

Transverse hippocampal slices (400 μ m) were obtained as previously described (Potier et al. 2000) from rats anesthetized with halothane before decapitation. Slices were prepared in ice-cold artificial cerebrospinal fluid (aCSF) and placed in a holding chamber for at least 1 h. The composition of aCSF was as follows (in mM): NaCl 124, KCl 3.5, MgSO₄ 1.5, CaCl₂ 2.3, NaHCO₃ 26.2,

NaH_2PO_4 1.2, and glucose 11, pH 7.4. A single slice was transferred to the recording chamber at a time and continuously perfused with aCSF pre-gassed with 95 % $\text{O}_2/5$ % CO_2 .

Extracellular recordings were obtained at 28–30 °C. Bipolar tungsten electrodes were used for stimulation, and 2–6-M Ω glass micropipettes were filled with 2 M NaCl for recording. Stimulation and recording electrodes were placed in the middle one third of the dentate gyrus molecular layer (500 μm apart). Presynaptic fiber volleys (PFVs) and field excitatory postsynaptic potentials (fEPSPs) were evoked, and the averaged slope of three PFVs and fEPSPs was measured in the linear part using Win LTP software (Anderson and Collingridge 2001). To evaluate the level of receptor activation, an index of synaptic efficacy (I_{SE}) corresponding to the fEPSP/PFV slopes ratio was plotted against stimulus intensity (500 to 900 μA). In some experiments, NMDA-R-mediated fEPSPs were isolated in slices perfused with low- Mg^{2+} (0.1 mM) aCSF supplemented with the non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX, 10 μM). The effects of exogenous D-serine added at 100 μM to saturate NMDA-R glycine-binding sites (Junjaud et al. 2006) were assessed by determining changes in I_{SE} 15 min after the addition of the co-agonist to the aCSF.

In order to investigate LTP of synaptic transmission, a test stimulus was applied every 15 s in aCSF supplemented for at least 10 min with 50 μM bicuculline methiodide (Tocris, Illkirch, France). This was necessary to shut down the powerful inhibition occurring in the dentate gyrus, which counteracts the induction of synaptic plasticity (Arima-Yoshida et al. 2011). The intensity was adjusted to obtain a fEPSP with a baseline slope of 0.1 V/s. The averaged slope of three fEPSPs was measured for 15 min before the delivery of a 100 Hz conditioning stimulus (1-s duration at the test intensity). Testing with a single pulse was then resumed for 60 min to determine the level of LTP.

In pharmacological experiments, all drugs were applied to the aCSF 10 min before the establishment of the baseline and maintained throughout the recording. The drugs applied included D-2-amino-5-phosphonovalerate (D-APV, 30–50 μM), D-serine (100 μM), glycine (500 μM) and the specific antagonist of the NMDA-R glycine-binding site L689,560 (10 μM) (all from Tocris, France).

In some experiments performed in adult animals, LTP was monitored in slices preincubated for at least 90 min with 0.4 U/ml (0.4 $\mu\text{g/ml}$) recombinant pure D-amino acid oxidase (DAAO, EC 1.4.3.3), the D-serine degrading enzyme, or with 20 $\mu\text{g/ml}$ purified recombinant D-serine deaminase (DsdA, EC 4.3.1.18) to remove endogenous D-serine. The purified recombinant enzymes were prepared as previously described (Fantinato et al. 2001; Shleper et al. 2005).

Data analysis

All results are expressed as means $\pm$ SEM. The significance of LTP expression was determined by comparing the 15 min of baseline recordings with values recorded between 45 and 60 min after the conditioning stimulation. The significance of changes in LTP magnitude between groups was determined by comparing the last 15 min of recordings. In order to take into account the correlations inherent in repeated measures (LTP and input/output curves), *p* values were calculated using multivariate analyses of variance followed by Tukey's post hoc tests. A paired *t* test was used to evaluate the significance of D-serine effects on NMDA synaptic transmission. In all cases, differences were considered significant when $p \leq 0.05$.

Results

D-serine contributes to NMDA-R-dependent LTP in the dentate gyrus

The action of D-serine on the NMDA-R glycine-binding site is required for the expression of synaptic plasticity in the hippocampal CA1 (Henneberger et al. 2010; Mothet et al. 2006; Yang et al. 2003), but its contribution to NMDA-R-dependent LTP processes in the dentate gyrus remains to be characterized (see Balu et al. 2013). In control aCSF containing bicuculline (see “Methods”), the 1 $\times$ 100 Hz conditioning stimulation delivered to slices from adult animals (23 slices from 21 rats) induced a significant potentiation of synaptic transmission [$F_{1,48}=33.7$, $p<0.0001$] that persisted until the end of the recording (Fig. 1). This LTP was dependent on the activation of NMDA-R glycine-binding sites since it was abolished in slices bathed with 50 μM of the selective competitive antagonist D-APV (nine slices from six rats) or with 10 μM of the selective antagonist

Fig. 1 Activation of NMDA-R glycine-binding site is necessary for LTP induction in the dentate gyrus. **a** Time course of mean 1×100 Hz-induced LTP calculated from slices ($n=23$) of young rats in a control aCSF and in medium supplemented with the NMDA-R competitive antagonist D-APV ($n=9$). **b** Time course of mean 1×100 Hz-induced LTP calculated from 23 slices of young rats in a control aCSF and from nine slices in medium supplemented with the competitive antagonist of the glycine binding site L689,560. For both **a** and **b**, in inserts are representative traces of fEPSPs recorded before and 60 min after the conditioning stimulation for each pharmacological condition. Bars: 10 ms and 0.5 mV. **c** Bar graphs illustrating the mean increase ($\pm$ SEM) in fEPSP slope averaged from 45 to 60 min after the conditioning stimulation in each pharmacological condition ($***p<0.0001$)

of the glycine-binding site L689,560 (nine slices from five rats) (Fig. 1), which did not affect basal synaptic transmission (Fig. S1A). The role of D-serine was then

determined in slices preincubated with the D-serine degrading enzyme DAAO to deplete endogenous D-serine content. While basal neurotransmission was not

modified (Fig. S1B), LTP was completely blocked in DAAO-pretreated tissues (nine slices from six rats) but was rescued by adding saturating concentrations of D-serine to the aCSF (eight slices from five rats) (Fig. 2a, c). These results suggest that LTP deficits in DAAO-treated slices could result from the lower occupancy of glycine-binding sites by the endogenous coagonist. However, treatment with DAAO generates hydrogen peroxide, known to affect LTP generation (Klann and Thiels 1999; Massaad et al. 2009; Serrano and Klann 2004). We therefore recorded LTP expression in slices preincubated with DsdA, a bacterial enzyme that catalyzes the deamination of D-serine into pyruvate and ammonia without generating H_2O_2 (Shleper et al. 2005). Similar to DAAO, treatment with DsdA did not affect basal synaptic transmission (Fig. S1C) but abolished LTP (nine slices from seven rats), and this was reversed by supplementing the aCSF with 500 μM glycine (11 slices from seven rats) (Fig. 2b). In these experiments, glycine was preferred to D-serine to reactivate the glycine-binding site to avoid fast degradation of added D-serine by DsdA.

Taken together, these results indicate that D-serine is necessary for LTP expression in the dentate gyrus, of adult animals as previously reported for the CA1 area (Balu et al. 2013; Henneberger et al. 2010; Mothet et al. 2006; Yang et al. 2003).

NMDA-R-dependent LTP is impaired in aged rats

In the next step, we attempted to determine whether LTP expression was impaired in the dentate gyrus of slices from aged animals under our experimental conditions.

In response to the conditioning stimulation, synaptic transmission immediately increased in slices from these animals (12 slices from 11 rats). However, this potentiation then slowly returned to normal, and 1 h after the tetanus, there was no statistical difference from baseline values [$F_{1,20}=0.86$, $p=0.23$]. Consequently, the magnitude of LTP was significantly decreased in aged animals when compared to adult controls [$F_{1,36}=17.5$, $p=0.0002$] (Fig. 3a).

We have shown above that LTP induction is closely dependent on NMDA-R activation. We therefore checked whether this activation was impaired in aged animals by isolating synaptic potentials specifically generated by these ionotropic glutamate receptors (see “Methods”).

Fig. 2 D-serine is required for LTP induction in the dentate gyrus of adult rats. **a** Time course of mean 1×100 Hz-induced LTP expressed in slices preincubated with the D-serine degrading enzyme D-amino acid oxidase (DAAO) with ($n=9$) or without ($n=9$) supplementation with D-serine (100 μM). **b** Time course of mean 1×100 Hz-induced LTP expressed in slices preincubated with the D-serine degrading enzyme D-serine deaminase (DsdA) with ($n=11$) or without ($n=9$) supplementation with glycine (500 μM). For both **a** and **b**, in inserts are representative traces of fEPSPs recorded before and 60 min after the conditioning stimulation for each enzymatic and pharmacological conditions. Bars: 10 ms and 0.5 mV. **c** Bar graphs illustrating the mean increase ($\pm$ SEM) in fEPSP slope averaged from 45 to 60 min after the conditioning stimulation in each pharmacological condition (* $p<0.05$ and ** $p<0.01$)

In slices from adult animals (11 slices from eight rats), PFVs and D-APV-sensitive fEPSPs were elicited by electrical stimulation (Fig. 3b). Interestingly, I_{SE} ,

Fig. 3 NMDA-R-dependent synaptic activation is impaired in the dentate gyrus of aged rats. **a** Time course of mean 1×100 Hz-induced LTP expressed in slices from adult ($n=23$) or aged rats ($n=12$). In inserts are representative traces of fEPSPs recorded before and 60 min after the conditioning stimulation (** $p<0.01$). Bars: 10 ms and 0.5 mV. **b** Superimposed samples traces of evoked NMDA-R-mediated fEPSPs recorded from a slice of an adult (left) and an aged rat (right) using a 600 μ A (1) or 800 μ A (2) current intensities and after addition of 30 μ M of the NMDA-R antagonist D-APV (3). **c** Comparison of NMDA-R-mediated synaptic efficacy defined as the fEPSP/PFV ratio calculated at stimulus intensity of 500 to 900 μ A in slices from adult ($n=11$) and aged rats ($n=12$) (* $p<0.05$)

determined by the fEPSP/PFV ratio, was significantly reduced in slices from aged animals (12 slices in six rats) regardless of the stimulus intensity [$F_{1,21}=6.1$, $p=0.02$] (Fig. 3c). Further analysis of the I_{SE} showed that the age

difference in PFV magnitude was not statistically significant [$F_{1,21}=3.3$, $p=0.08$] whereas fEPSPs were significantly diminished [$F_{1,21}=15.1$, $p=0.0008$], indicating a predominant contribution of postsynaptic mechanisms to the age-related impairment of NMDA-R activation.

Age-related deficits in NMDA-R activation and LTP are independent of changes at the glycine-binding site

Since the impaired activation of glycine-binding sites by endogenous D-serine, previously seen in the CA1 area (Mothet et al. 2006; Potier et al. 2010), could be a putative postsynaptic mechanism, we determined the responsiveness of isolated NMDA-R-mediated synaptic responses to the exogenous application of a 100 μ M saturating concentration of D-serine (Junjaud et al. 2006) in slices from both groups of animals.

In slices from adult rats (11 tested slices from seven rats), D-serine significantly increased the I_{SE} by around 30 % (paired t test, $p<0.01$), regardless of the stimulus intensity (Fig. 4a), indicating that glycine-binding sites are not saturated in dentate gyrus slice preparations. In contrast, recordings performed in slices from aged animals (12 slices from six rats) showed that the exogenous application of the NMDA-R co-agonist was unable to significantly enhance the I_{SE} , except at the highest intensities of stimulation (Fig. 4a), suggesting that glycine-binding sites become saturated by endogenous D-serine with age in this particular hippocampal area. An age-related alteration of the NMDA-R subunit composition could also contribute to this saturation effect (Bai et al. 2004) that seems unlikely since the affinity of the GluN2 versus GluN1 subunits for D-serine is quite similar (Matsui et al. 1995; Priestley et al. 1995). Since the I_{SE} remains significantly weaker in aged animals than in adults under conditions of saturating D-serine (Fig. 4b), one could argue that changes in glycine-binding-site occupancy do not underlie the age-related deficits in NMDA-R-dependent synaptic potentials in the dentate gyrus.

We next checked the effects of exogenous D-serine on the age-related impairment of LTP expression. When delivered in the presence of 100 μ M D-serine, significant LTP [$F_{1,25}=12.6$, $p=0.001$] was induced in slices from adult rats (14

Fig. 4 Age-related deficits of NMDA-R synaptic activation do not depend on D-serine. **a** Effects of D-serine (100 μ M) on NMDA-R-mediated synaptic efficacy calculated at stimulus intensity of 500 to 900 μ A in slices from adult (left, $n=11$) and aged rats (right, $n=12$) ($**p<0.01$). **b** Comparison of NMDA-R-mediated synaptic efficacy in slices from adult and aged rats calculated in the presence of D-serine (100 μ M) ($**p<0.01$). **c** Time course of mean 1×100 Hz-induced LTP expressed in slices from adult ($n=14$) or aged rats ($n=11$) supplemented with D-serine (100 μ M). In inserts are representative traces of fEPSPs recorded before and 60 min after the conditioning stimulation ($**p<0.01$). Bars: 10 ms and 0.5 mV

slices from nine rats), reaching a magnitude similar to that obtained with control aCSF [$F_{1,40}=0.1$, $p=0.77$]. In aged rats (11 slices from eight rats), providing D-serine to slices did not modify the response to the tetanus and no long-lasting potentiation was generated (Fig. 4c). Consequently, the age-related LTP impairment was maintained in the dentate gyrus even after saturating NMDA-R glycine-binding sites with D-serine [$F_{1,22}=8.5$, $p=0.008$]. A similar result was obtained by using exogenous glycine (500 μ M) to activate glycine-binding sites (six slices from three animals) (not illustrated).

Presynaptic mechanisms participate in age-related deficits of basal glutamatergic synaptic transmission

We have postulated above that the age-related impairment of NMDA-R activation occurs predominantly through postsynaptic mechanisms, based on the fact that PFVs elicited by the stimulation of glutamate afferents are not statistically modified in aged animals whereas fEPSPs generated at the postsynaptic level are significantly reduced. However, the possibility of a presynaptic contribution could not be definitively ruled out, considering that extracellular recordings of isolated NMDA-R-dependent potentials require low- Mg^{2+}

aCSF, which greatly alters fiber excitability. This experimental constraint could therefore have masked a putative age-related difference in PFVs, which, although not significant, was close to the threshold for biological relevance ($p=0.08$). We therefore attempted to overcome this bias by recording synaptic potentials in the dentate gyrus in control aCSF.

In adult rat slices (35 slices from 17 rats), PFVs and fEPSPs were elicited by electrical stimulation (Fig. 5a), the latter being blocked by NBQX at the end of the recording. Under the same conditions, PFV slopes were significantly reduced in aged animals [$F_{1,73}=12.6$, $p=0.0008$] (33 slices in 12 rats), indicating that presynaptic responsiveness is indeed altered in the aged rat dentate gyrus (Fig. 5b). Interestingly, non-NMDA-mediated fEPSPs were reduced to a similar extent [$F_{1,73}=11.6$, $p=0.0008$] (Fig. 5c), and the I_{SE} was maintained in aged individuals (Fig. 5d). These results strongly suggest that the principal contribution to the age-related decrease in basal glutamate transmission in the dentate gyrus comes from a presynaptic defect.

Discussion

The present study provides new and additional evidence showing that the relative importance of the various cellular mechanisms underlying age-related deficits in hippocampus-dependent memory presents clear-cut regional differences, at least with respect to the functional properties of neuronal networks in the CA1 and dentate gyrus. Conceptually, this is not surprising, considering the specificity of the cell types and connectivity of each hippocampal region. However, the fact that glutamate transmission and NMDA-R activation mainly underlie neuronal communication and synaptic plasticity within both areas raises the possibility of common alterations during the process of aging. Our results indicate that the D-serine-dependent regulation of NMDA-R activation is necessary for LTP expression in the dentate gyrus but is not a crucial mechanism underlying age-related deficits, which depend more on changes in presynaptic afferent activity and postsynaptic receptor density. These results are in clear contrast to the predominant role of the D-serine-related pathway in the LTP deficits that take place

Fig. 5 Changes in pre-synaptic activity underlies the age-related decrease in basal synaptic transmission. **a** Superimposed samples traces of evoked non-NMDA-R-mediated fEPSPs recorded in a slice from an adult (*top*) and an aged rat (*bottom*) before and after supplementation with the non-NMDAR antagonist NBQX (10 μM). **b** Plot of the mean PFV slope against the stimulus

intensity calculated from slices of adult ($n=35$) and aged ($n=35$) rats. (** $p<0.01$). **c** Plot of the mean fEPSP slope against the stimulus intensity calculated from slices of the same animals. (** $p<0.01$). **d** Comparison of non-NMDA-R-mediated synaptic efficacy (I_{SE}) determined in slices from adult and aged rats for 300, 400, and 500 μA stimulus intensities.

during aging in the CA1 area (Haxaire et al. 2012; Junjaud et al. 2006; Mothet et al. 2006; Turpin et al. 2012), in which a significant contribution of presynaptic factors has been ruled out (see Burke and Barnes 2010 for review). These results therefore highlight the importance of the regional specificity of the cellular and functional alterations underlying age-related memory deficits, even at the level of a single structure such as the hippocampal formation, a major point to be taken into consideration in therapeutic strategies aimed at reducing cognitive aging.

After several decades of intensive investigations, there is now no doubt that the weaker capacity of aging neuronal networks to adapt their synaptic communication to changes in environmental conditions underlies cognitive defects, particularly memory deficits (Rosenzweig and Barnes 2003). In view of its pivotal role in memory formation, most aging studies have investigated changes in activity in the hippocampus, focusing more intensively on the CA1 area than the dentate gyrus (see Barnes 2003; Billard 2006; Burke and Barnes 2010; Foster 2006; Lynch 2010 for reviews). Taken together, these studies show that age-related deficits in synaptic plasticity affect the entire hippocampus, but whether the different hippocampal areas share the same pathways has remained unresolved (Barnes 1994). This is particularly the case with the impaired LTP seen in the CA1 area as well as in the dentate gyrus of the aging hippocampus, since changes in receptor density or pharmacological properties have mostly been studied in the former (Barnes et al. 1996, 1997; Potier et al. 2000) while the impact of cytokines has mainly been investigated in the latter (see Lynch 2010).

It has recently been found that the decrease in levels of the endogenous NMDA-R co-agonist D-serine is a critical mechanism driving LTP impairment in the CA1 of aged rats (Haxaire et al. 2012; Mothet et al. 2006; Turpin et al. 2011), indicating a possible role for the amino acid in the age-related deficits occurring in the dentate gyrus also. We show in this report that degrading endogenous D-serine through tissue incubation with DAAO impairs LTP expression, although this could be due to the production of reactive oxygen species (ROS) during degradation. However, LTP impairment by DAAO can be rescued by providing D-serine to the external medium. In addition, LTP is also compromised by DsdA, which selectively degrades D-serine without ROS production (Shleper et al. 2005). From these

results, we can conclude that the activation of NMDA-R by D-serine is indeed required for LTP expression in the dentate gyrus. Such a critical role for the amino acid at this particular hippocampal location has also been recently characterized in mice knocked out for the synthesizing enzyme serine racemase, which display a 90 % reduction in cortical D-serine (Balu et al. 2013). However, a contribution of glycine cannot be definitively ruled out, since this amino acid may be significantly involved when synaptic activation is forced, such as in the LTP paradigm, as recently demonstrated in the amygdala (Li et al. 2013). Indeed, preliminary results from our group indicate that LTP is also impaired in the dentate gyrus in slices incubated with glycine oxidase, which specifically removes glycine (Billard and Wolosker, unpublished results). Nevertheless, a role for this amino acid in age-related LTP deficits is unlikely, since glycine levels do not change with age in hippocampal tissues (Mothet et al. 2006). In addition, saturating NMDA-R co-agonist binding sites with exogenous glycine does not rescue the impaired LTP displayed by aged animals. Importantly, our results also indicate that D-serine does not play a major role in these aged-related deficits, since neither impaired LTP nor reduced NMDA-R activation is reversed by providing the D-amino acid to tissues, in striking contrast to what has been found in the CA1 area (Potier et al. 2010). These results therefore indicate that the putative consequences of age-related changes in the activation of NMDA-R co-agonist binding sites are minimal in the dentate gyrus, compared to the importance of these alterations in CA1 neuronal networks.

Ours result indicate that isolated NMDA-R-dependent synaptic responses decrease with age in the dentate gyrus, as reported by previous extracellular and patch-clamp recording studies (Krause et al. 2008; Yang et al. 2008). This impairment was observed under specific experimental conditions of exacerbated excitability (through Mg^{2+} omission from the aCSF) in which the activation of presynaptic glutamate afferents was similar in adult and aged subjects, as well as under conditions of maximal NMDA-R activation where co-agonist binding sites were saturated with D-serine. Thus, the decrease in NMDA-R density, previously reported in the dentate gyrus of aged rodents and monkeys (Gazzaley et al. 1996; Magnusson et al. 2002; Morrison and Gazzaley 1996; Newton et al. 2007), significantly contributes to the reduction in NMDA-R activation. On the other

hand, our results also indicate an age-related decrease in the magnitude of presynaptic afferent activity when synaptic responses are recorded under control conditions, as previously reported (Barnes et al. 1992; Burke et al. 2012; Yang et al. 2008). Therefore, in addition to a lower postsynaptic receptor density, the age-related decrease in NMDA-R activation also reflects weaker glutamate release consecutive to a reduction in synaptic contacts from axons originating in the entorhinal cortex (Geinisman et al. 1992; Nicholson et al. 2004; Smith et al. 2000). It is interesting to note that in our study, the decrease in the size of PFVs paralleled a similar reduction in postsynaptic potentials. This result differs from earlier data indicating that the loss of afferent synapses in the dentate gyrus during senescence is compensated for by an increase in the quantal size of the remaining synaptic inputs such that the PFV/fEPSP ratio is larger in aged animals (Barnes and McNaughton 1980; Foster et al. 1991; Yang et al. 2008). While this discrepancy could reflect differences in rat strains and/or technical protocols, our present results are validated by patch-clamp recordings showing that dendritic integration is altered in the granule cells of aged rats, resulting in weaker excitatory postsynaptic currents (Krause et al. 2008).

Besides the role of presynaptic inputs, the results of our present study also suggest that the reduced NMDA-R density could mask a possible role for D-serine. Indeed, levels of the amino acid are reported to be reduced in extracts of the whole aging hippocampus (Mothet et al. 2006; Turpin et al. 2011), and preliminary data indicating that the expression of the D-serine synthesizing enzyme serine racemase is also reduced in the dentate gyrus during aging (Latour and Denis, personal communication). We could thus speculate that despite their age-related decrease, D-serine levels are still elevated enough to saturate the low density of NMDA-R expressed in the dentate gyrus at this time (Gazzaley et al. 1996; Magnusson et al. 2002; Morrison and Gazzaley 1996; Newton et al. 2007). This could explain why providing exogenous D-serine does not enhance NMDA-R activation at this location as it does in the CA1 of aged animals (Haxaire et al. 2012; Junjaud et al. 2006; Mothet et al. 2006; Turpin et al. 2011).

To conclude, this study provides additional viewpoints concerning the complexity of changes in circuit connectivity and functionality underlying hippocampal aging and particularly the relative contribution of each of these changes with regard to the subregion considered

(see also Burke and Barnes 2010). We are still far from understanding the respective importance of the various specific alterations and how they interact, but the fact that relieving each of these deficits individually is capable of reversing age-related memory impairment, as described in the literature for different processes, indicates that they all affect cognitive performance.

Acknowledgment This manuscript was prepared with editorial help from Gap Junction (www.gap-junction.com). This work was supported by the National Institute of Health and Medical research (J-M.B) and by the Israel Science Foundation, Legacy Heritage Fund and the Allen and Jewel Prince Center for Neurodegenerative Disorders of the brain (H.W)

Conflict of interest All authors state that they have no actual or potential conflicts of interest, including financial, personal, or other relationships with concerned people or organizations within 3 years of beginning the work reported here.

References

- Anderson WW, Collingridge GL (2001) The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *J Neurosci Methods* 108(1):71–83
- Arima-Yoshida F, Watabe AM, Manabe T (2011) The mechanisms of the strong inhibitory modulation of long-term potentiation in the rat dentate gyrus. *Eur J Neurosci* 33(9):1637–1646
- Bai L, Hof PR, Standart DG, Xing Y, Nelson SE, Young AB, Magnusson KR (2004) Changes in the expression of the NR2B subunit during aging in macaque monkeys. *Neurobiol Aging* 25(2):201–208
- Balu DT, Li Y, Puhl MD, Benneyworth MA, Basu AC, Takagi S, Bolshakov VY, Coyle JT (2013) Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. *Proc Natl Acad Sci USA*
- Barnes CA (1994) Normal aging: regionally specific changes in hippocampal synaptic transmission. *Trends Neurosci* 17(1):13–18
- Barnes CA (2003) Long-term potentiation and the ageing brain. *Philos Trans R Soc Lond B Biol Sci* 358(1432):765–772
- Barnes CA, McNaughton BL (1980) Physiological compensation for loss of afferent synapses in rat hippocampal granule cells during senescence. *J Physiol* 309:473–485
- Barnes CA, McNaughton BL (1985) An age comparison of the rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. *Behav Neurosci* 99(6):1040–1048
- Barnes CA, Rao G, Foster TC, McNaughton BL (1992) Region-specific age effects on AMPA sensitivity: electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. *Hippocampus* 2(4):457–468
- Barnes CA, Rao G, Houston FP (2000) LTP induction threshold change in old rats at the perforant path—granule cell synapse. *Neurobiol Aging* 21(5):613–620

- Barnes CA, Rao G, McNaughton BL (1996) Functional integrity of NMDA-dependent LTP induction mechanisms across the lifespan of F-344 rats. *Learn Mem* 3(2–3):124–137
- Barnes CA, Rao G, Shen J (1997) Age-related decrease in the N-methyl-D-aspartate R-mediated excitatory postsynaptic potential in hippocampal region CA1. *Neurobiol Aging* 18(4):445–452
- Billard JM (2006) Ageing, hippocampal synaptic activity and magnesium. *Magn Res* 19(3):199–215
- Billard JM (2012) D-amino acids in brain neurotransmission and synaptic plasticity. *Amino Acids* 43(5):1851–1860
- Burke SN, Barnes CA (2010) Senescent synapses and hippocampal circuit dynamics. *Trends Neurosci* 33(3):153–161
- Burke SN, Ryan L, Barnes CA (2012) Characterizing cognitive aging of recognition memory and related processes in animal models and in humans. *Front Aging Neurosci* 4:15
- Clayton DA, Mesches MH, Alvarez E, Bickford PC, Browning MD (2002) A hippocampal NR2B deficit can mimic age-related changes in long-term potentiation and spatial learning in the Fischer 344 rat. *J Neurosci* 22(9):3628–3637
- Collingridge GL, Bliss TV (1995) Memories of NMDA receptors and LTP. *Trends Neurosci* 18(2):54–56
- Diana G, Scotti de Carolis A, Frank C, Domenici MR, Sagratella S (1994) Selective reduction of hippocampal dentate frequency potentiation in aged rats with impaired place learning. *Brain Res Bull* 35(2):107–111
- Dieguez D Jr, Barea-Rodriguez EJ (2004) Aging impairs the late phase of long-term potentiation at the medial perforant path-CA3 synapse in awake rats. *Synapse* 52(1):53–61
- Fantinato S, Pollegioni L, Pilone MS (2001) Engineering, expression and purification of a His-tagged chimeric D-amino acid oxidase from *Rhodotorula gracilis*. *Enzym Micro Technol* 29:407–412
- Foster TC (2002) Regulation of synaptic plasticity in memory and memory decline with aging. *Prog Brain Res* 138:283–303
- Foster TC (2006) Biological markers of age-related memory deficits: treatment of senescent physiology. *CNS Drugs* 20(2):153–166
- Foster TC, Barnes CA, Rao G, McNaughton BL (1991) Increase in perforant path quantal size in aged F-344 rats. *Neurobiol Aging* 12(5):441–448
- Gazzaley AH, Siegel SJ, Kordower JH, Mufson EJ, Morrison JH (1996) Circuit-specific alterations of N-methyl-D-aspartate receptor subunit 1 in the dentate gyrus of aged monkeys. *Proc Natl Acad Sci U S A* 93(7):3121–3125
- Geinisman Y, deToledo-Morrell L, Morrell F, Persina IS, Rossi M (1992) Structural synaptic plasticity associated with the induction of long-term potentiation is preserved in the dentate gyrus of aged rats. *Hippocampus* 2(4):445–456
- Griffin R, Nally R, Nolan Y, McCartney Y, Linden J, Lynch MA (2006) The age-related attenuation in long-term potentiation is associated with microglial activation. *J Neurochem* 99(4):1263–1272
- Haxaire C, Turpin FR, Potier B, Kervern M, Sinet PM, Barbanel G, Mothet JP, Dutar P, Billard JM (2012) Reversal of age-related oxidative stress prevents hippocampal synaptic plasticity deficits by protecting d-serine-dependent NMDA receptor activation. *Aging Cell* 11(2):336–344
- Henneberger C, Papouin T, Oliet SH, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463(7278):232–236
- Izquierdo I (1991) Role of NMDA receptors in memory. *Trends Pharmacol Sci* 12(4):128–129
- Junjaud G, Rouaud E, Turpin F, Mothet JP, Billard JM (2006) Age-related effects of the neuromodulator D-serine on neurotransmission and synaptic potentiation in the CA1 hippocampal area of the rat. *J Neurochem* 98(4):1159–1166
- Kim SJ, Linden DJ (2007) Ubiquitous plasticity and memory storage. *Neuron* 56(4):582–592
- Klann E, Thiels E (1999) Modulation of protein kinases and protein phosphatases by reactive oxygen species: implications for hippocampal synaptic plasticity. *Prog Neuropsychopharmacol Biol Psychiatry* 23(3):359–376
- Krause M, Yang Z, Rao G, Houston FP, Barnes CA (2008) Altered dendritic integration in hippocampal granule cells of spatial learning-impaired aged rats. *J Neurophysiol* 99(6):2769–2778
- Li Y, Sacchi S, Pollegioni L, Basu AC, Coyle JT, Bolshakov VY (2013) Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. *Nat Commun* 4:1760
- Lisman JE, McIntyre CC (2001) Synaptic plasticity: a molecular memory switch. *Curr Biol* 11(19):R788–R791
- Luo L, Craik FI (2008) Aging and memory: a cognitive approach. *Can J Psychiatry* 53(6):346–353
- Lynch MA (1998) Analysis of the mechanisms underlying the age-related impairment in long-term potentiation in the rat. *Rev Neurosci* 9(3):169–201
- Lynch MA (2004) Long-term potentiation and memory. *Physiol Rev* 84(1):87–136
- Lynch MA (2010) Age-related neuroinflammatory changes negatively impact on neuronal function. *Front Aging Neurosci* 1:6
- Magnusson KR, Nelson SE, Young AB (2002) Age-related changes in the protein expression of subunits of the NMDA receptor. *Brain Res Mol Brain Res* 99(1):40–45
- Malenka RC, Nicoll RA (1993) NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci* 16(12):521–527
- Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* 23:649–711
- Massaad CA, Washington TM, Pautler RG, Klann E (2009) Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 106(32):13576–13581
- Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K (1995) Functional comparison of D-serine and glycine in rodents: the effects on cloned NMDA receptors and the extracellular concentration. *J Neurochem* 65(1):454–458
- Moore CI, Browning MD, Rose GM (1993) Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. *Hippocampus* 3(1):57–66
- Morrison JH, Gazzaley AH (1996) Age-related alterations of the N-methyl-D-aspartate receptor in the dentate gyrus. *Mol Psychiatry* 1(5):356–358
- Mothet JP, Rouaud E, Sinet PM, Potier B, Jouvenceau A, Dutar P, Videau C, Epelbaum J, Billard JM (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. *Aging Cell* 5(3):267–274

- Newton IG, Forbes ME, Linville MC, Pang H, Tucker EW, Riddle DR, Brunso-Bechtold JK (2007) Effects of aging and caloric restriction on dentate gyrus synapses and glutamate receptor subunits. *Neurobiol Aging*
- Nicholson DA, Yoshida R, Berry RW, Gallagher M, Geinisman Y (2004) Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. *J Neurosci* 24(35):7648–7653
- Potier B, Poindessous-Jazat F, Dutar P, Billard JM (2000) NMDA receptor activation in the aged rat hippocampus. *Exp Gerontol* 35(9–10):1185–1199
- Potier B, Turpin FR, Sinet PM, Rouaud E, Mothet JP, Videau C, Epelbaum J, Dutar P, Billard JM (2010) Contribution of the D-serine-dependent pathway to the cellular mechanisms underlying cognitive aging. *Front Aging Neurosci* 2:1
- Priestley T, Laughton P, Myers J, Le Bourdelles B, Kerby J, Whiting PJ (1995) Pharmacological properties of recombinant human N-methyl-D-Aspartate receptors comprising NR1a/NR2A and NR1a/NRB subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol Pharmacol* 48(5):841–848
- Rosenzweig ES, Barnes CA (2003) Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. *Prog Neurobiol* 69(3):143–179
- Serrano F, Klann E (2004) Reactive oxygen species and synaptic plasticity in the aging hippocampus. *Ageing Res Rev* 3(4):431–443
- Shankar S, Teyler TJ, Robbins N (1998) Aging differentially alters forms of long-term potentiation in rat hippocampal area CA1. *J Neurophysiol* 79(1):334–341
- Shleper M, Kartvelishvily E, Wolosker H (2005) D-serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J Neurosci* 25(41):9413–9417
- Sierra-Mercado D, Dieguez D Jr, Barea-Rodriguez EJ (2008) Brief novelty exposure facilitates dentate gyrus LTP in aged rats. *Hippocampus* 18(8):835–843
- Smith TD, Adams MM, Gallagher M, Morrison JH, Rapp PR (2000) Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. *J Neurosci* 20(17):6587–6593
- Turpin F, Dallerac G, Mothet JP (2012) Electrophysiological analysis of the modulation of NMDA-receptors function by D-serine and glycine in the central nervous system. *Methods Mol Biol* 794:299–312
- Turpin FR, Potier B, Dulong JR, Sinet PM, Alliot J, Oliet SH, Dutar P, Epelbaum J, Mothet JP, Billard JM (2011) Reduced serine racemase expression contributes to age-related deficits in hippocampal cognitive function. *Neurobiol Aging* 32(8):1495–1504
- Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S (2003) Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc Natl Acad Sci U S A* 100(25):15194–15199
- Yang Z, Krause M, Rao G, McNaughton BL, Barnes CA (2008) Synaptic commitment: developmentally regulated reciprocal changes in hippocampal granule cell NMDA and AMPA receptors over the lifespan. *J Neurophysiol* 99(6):2760–2768