Impairments of Synaptic Plasticity in Aged Animals and in Animal Models of Alzheimer's Disease

Marta Balietti,^{1,2} Francesco Tamagnini,³ Patrizia Fattoretti,^{1,2} Costanza Burattini,³ Tiziana Casoli,¹ Daniela Platano,⁴ Fabrizia Lattanzio,⁵ and Giorgio Aicardi^{3,6}

Abstract

Aging is associated with a gradual decline in cognitive functions, and more dramatic cognitive impairments occur in patients affected by Alzheimer's disease (AD). Electrophysiological and molecular studies performed in aged animals and in animal models of AD have shown that cognitive decline is associated with significant modifications in synaptic plasticity (*i.e.*, activity-dependent changes in synaptic strength) and have elucidated some of the cellular mechanisms underlying this process. Morphological studies have revealed a correlation between the quality of memory performance and the extent of structural changes of synaptic contacts occurring during memory consolidation. We briefly review recent experimental evidence here.

Introduction

DESPITE IMPORTANT ADVANCES IN MEDICINE and large financial investments, the neurobiological bases of cognitive decline occurring during aging and neurodegenerative diseases are not fully understood. Studies performed in aged animals and in animal models of Alzheimer's disease (AD) have revealed that deterioration of cognitive performances is associated with significant changes in synaptic plasticity, *i.e.*, activity-dependent changes in synaptic strength underlying memory formation. In particular, electrophysiological studies have documented that impairments of long-term declarative memory, especially vulnerable to age and neurodegenerative diseases, are associated with alterations of long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus and other regions of the temporal lobe involved in this form of memory; the thresholds for LTP and LTD induction are generally increased and decreased, respectively (for review, see Kumar¹).

Age- and AD-Related Changes in LTP

Recent observations indicate that age-related hippocampal glutamatergic LTP impairment is in part due to insufficient postsynaptic depolarization deriving from a decrease in the *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic

response and an increase in after-hyperpolarization (AHP) amplitude. The decline in NMDA receptor responsiveness may be due to age-related alterations in: (1) calcium (Ca^{2+}) homeostasis, leading to increased activity of the phosphatase calcineurin, which in turn reduces Ca^{2+} influx through the receptor,² and (2) redox state (decrease in redox buffering capacity), leading to oxidation of extracellular cysteine residues on the receptor³ and to a decline in Ca^{2+} -calmodulin-dependent protein kinase II (CaMKII) activity.⁴ The involvement of AHP is supported by the observation that pharmacological manipulations that reduce AHP amplitude, such as blockade of L-type Ca^{2+} channels, inhibition of Ca^{2+} release from intracellular stores (which leads to a decreased activation of Ca^{2+} -activated potassium [K^+] channels), or direct blockade of K^+ channels, also overcome the age-related increase of threshold for LTP induction.⁵ The age-related increase of microglia and astrocyte activation may also play a significant role in LTP impairment, because rosiglitazone⁶ and the ω -3 polyunsaturated fatty acid docosapentaenoic acid⁷ are capable of attenuating the effect of age on LTP.

Concerning AD, evidence indicates that the progressive accumulation of soluble amyloid- β ($\text{A}\beta$) peptides contributes to LTP impairment through interaction with different intracellular signaling pathways, including phosphatase calcineurin, protein phosphatase 1, cyclic adenosine monophosphate/protein kinase A (cAMP/PKA), cAMP response

¹Neurobiology of Aging Laboratory and ²Cellular Bioenergetics Laboratory, INRCA Scientific Technological Area, Ancona, Italy.

³Department of Human and General Physiology, University of Bologna, Bologna, Italy.

⁴Laboratory of Immunorheumatology and tissue regeneration, Istituto Ortopedico Rizzoli, Bologna, Italy.

⁵Scientific Direction, INRCA Scientific Technological Area, Ancona, Italy.

⁶Interdepartmental Centre "Luigi Galvani" for the Study of Biophysics, Bioinformatics and Biocomplexity, University of Bologna, Bologna, Italy.

element-binding (CREB) protein, and CaMKII⁸ (for review, see Kumar¹). Apolipoprotein E (ApoE) is a lipid transport protein that participates in the regulation of plasma cholesterol and in the transport of dietary lipids. It exists in three isoforms (E2, E3, and E4), and the majority of the human population is homozygous for ApoE3; ApoE4 is present in about 16% of the population and is associated with a significantly earlier average age of onset of dementia, whereas the rare ApoE2 form is protective. Differential effects of ApoE isoforms on A β clearance and cytoskeleton stability have been reported.⁹ Recent studies also demonstrate that ApoE4 is directly involved in AD-related impairment of synaptic plasticity. For instance, ApoE4 versus ApoE3/2 knock-in mice show impaired reelin-mediated NMDA receptor phosphorylation and LTP enhancement. ApoE4 reduces the neuronal surface expression, via increased internalization, of ApoER2 (a dual function receptor for ApoE and reelin), NMDA and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, thereby critically reducing reelin-mediated enhancement of glutamatergic neurotransmission.¹⁰

Age- and AD-Related Changes in LTD

As mentioned above, the increase in threshold for LTP induction observed in aging and AD is generally associated with a decrease in threshold for LTD induction (for review, see Kumar¹). This might simply reflect a shift in the frequency-response function (obtained by plotting the change in synaptic strength as a result of different conditioning stimulation frequencies¹¹), which is based on intracellular Ca²⁺ levels: (1) a high increase of frequency (e.g., 100 Hz) from the basal stimulation rate results in the fast and robust increase of intracellular Ca²⁺ which activates Ca²⁺-dependent kinases leading to LTP, whereas (2) a smaller increase (e.g., 1–5 Hz) results in a slower and less prominent Ca²⁺ rise, leading to LTD through the activation of Ca²⁺-dependent phosphatases. Thus, the same mechanisms (summarized above) responsible for age- and AD-related increase in LTP threshold might result in decrease in LTD threshold because they induce a shift in the frequency-response function. In contrast to this hypothesis, we have recently found that basal synaptic transmission and 5-Hz-induced LTD are impaired in perirhinal cortex slices obtained from 3 month-old Tg2576 mice (one of the most studied AD transgenic models).¹² This observation is consistent with visual recognition memory deficits observed in these mice at 5 months of age and in AD patients in the very early phase of the disease, because LTD in this cortical area is regarded as the cellular correlate of this form of memory (for references, see Tamagnini et al.¹²). A recent study has shown that A β may induce synaptic depression by employing signaling pathways of LTD to increase endocytosis of AMPA synaptic receptors and to decrease spine density; this may result in a partial occlusion of LTD,¹³ as observed in our experiments.

Age- and AD-Related Changes in Brain-Derived Neurotrophic Factor–trkB Signaling

Long-term memory formation is based on stable modifications of synaptic strength, which require new synthesis and increase in secretion of brain-derived neurotrophic factor (BDNF),^{14,15} which in turn interacts with its receptor trkB, leading to relevant morphological changes (remodeling) of

synaptic contacts.¹⁶ Several investigations in humans and experimental animal models have shown a decline in BDNF and trkB expression in the hippocampus and in the neocortex during physiological aging and AD (for review, see Tapia-Arancibia et al.¹⁷). Interestingly, recent findings show that some physiological or pathological age-associated changes in the central nervous system could be offset by administration of exogenous BDNF and/or by stimulating its receptor expression or activation¹⁸; these studies might lead to therapeutic treatments to enhance healthy aging (for review, see Fumagalli et al.¹⁹).

Age- and AD-Related Synaptic Structural Changes

In parallel with the electrophysiological and molecular approach, electron microscopy studies have documented age- and AD-related structural changes in the brain structures involved in memory formation, including enlargement and loss of synaptic contacts, decrease of the size of perforated postsynaptic densities, and altered mitochondrial dynamics (for review, see Bertoni-Freddari et al.²⁰ and Reddy et al.²¹). However, these studies consist in a “static” morphological evaluation of the aged or the AD brain (differences vs. the adult or the normal aged brain, respectively), and little is known about age- and AD-related differences in synaptic remodeling (“dynamic” evaluation) occurring during memory consolidation.

In a recent study, we found that object recognition visual training able to induce long-term memory formation in aged (25- to 27-month-old) rats is associated with a more marked structural synaptic remodeling in layer II of the perirhinal cortex than that induced by the same training in adult (4- to 6-month-old) rats, possibly reflecting a preexisting lower dynamic status.²² To investigate cellular and structural correlates of the different quality of memory performance observed among aged individuals, in the following studies we compared synaptic remodeling and gene expression in the hippocampus of aged (26- to 27-month-old) rats trained with the inhibitory avoidance task, which is capable of providing evidence of diverse long-term spatial-associative memory performances. We found that better-performing animals were characterized by a more efficient plastic remodeling of synaptic contacts and mitochondria in the stratum moleculare of the CA1 region (9 hr after the memory task), suggesting that the preservation of structural plasticity during aging is critical for maintaining the ability to consolidate spatial memory traces.²³ Moreover, microarray analyses and quantitative real-time PCR measures allowed us to identify 61 genes expressed differentially expressed in the hippocampus of good versus bad performers, including genes functionally linked to the regulation of cytoskeleton and neurosignaling pathways.²⁴ We also provided the first evidence that: (1) Dynamin-binding protein (DMNBP, a scaffold protein regulating cytoskeletal actin microfilaments and synaptic vesicle pools, whose expression is low in neuropathologically confirmed AD patient's brains) messenger RNA (mRNA) expression is reduced in the rat hippocampus during physiological aging, and (2) good performers show a higher learning-induced increase of hippocampal DNMBP mRNA expression.²⁵

Morphological studies of synaptic contacts have also been performed to characterize the effect of nutritional approaches aimed at decreasing the detrimental effects of aging on cognition. Ketogenic diets (KDs), successfully used in the

therapy of pediatric epilepsy for nearly a century, have recently shown beneficial effects in neurodegeneration (for review, see Biliotti et al.²⁶). However, we found that KDs may exert even opposite effects on the synaptic structure in different brain regions, presumably depending on the different neuronal vulnerability to age among different brain areas. Only the cells that maintain a good functional profile during aging may be able to reactivate ketone bodies uptake and catabolism, allowing exploitation of the beneficial properties of KDs^{27–29} (for review, see Biliotti et al.²⁶).

Acknowledgments

The work was supported by the FIRB grant RBNE018R89-002 from the Italian Ministry of Education, University and Research, by the RFO grant from the University of Bologna, and by a fundamental contribution from the “Fondazione Cassa di Risparmio di Bologna” and the “Fondazione del Monte di Bologna e Ravenna”.

Author Disclosure Statement

No competing financial interests exist.

References

- Kumar A. Long-term potentiation at CA3-CA1 hippocampal synapses with special emphasis on aging, disease, and stress. *Front Aging Neurosci* 2011;3:7.
- Foster TC, Sharrow KM, Masse JR, Norris CM, Kumar A. Calcineurin links Ca^{2+} dysregulation with brain aging. *J Neurosci* 2001;21:4066–4073.
- Lipton SA, Choi YB, Takahashi H, Zhang D, Li W, Godzik A, Bankston LA. Cysteine regulation of protein function—as exemplified by NMDA-receptor modulation. *Trends Neurosci* 2002;25:474–480.
- Bodhinathan K, Kumar A, Foster TC. Intracellular redox state alters NMDA receptor response during aging through Ca^{2+} /calmodulin-dependent protein kinase II. *J Neurosci* 2010;30:1914–1924.
- Norris CM, Halpain S, Foster TC. Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca^{2+} channels. *J Neurosci* 1998;18:3171–3179.
- Cowley TR, O'Sullivan J, Blau C, Deighan BF, Jones R, Kerskens C, Richardson JC, Virley D, Upton N, Lynch MA. Rosiglitazone attenuates the age-related changes in astrogliosis and the deficit in LTP. *Neurobiol Aging* 2011;32:2318.e1–2318.e15.
- Kelly L, Grehan B, Chiesa AD, O'Mara SM, Downer E, Sahyoun G, Massey KA, Nicolaou A, Lynch MA. The polyunsaturated fatty acids, EPA and DPA exert a protective effect in the hippocampus of the aged rat. *Neurobiol Aging* 2011b; doi:10.1016/j.neurobiolaging.2010.04.001.
- Zeng Y, Zhao D, Xie CW. Neurotrophins enhance CaMKII activity and rescue amyloid-beta-induced deficits in hippocampal synaptic plasticity. *J Alzheimers Dis* 2010;21:823–831.
- Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: A causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci USA* 2006;103:5644–5651.
- Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci USA* 2010;107:12011–12016.
- Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: Orientation specificity and binocular interaction in visual cortex. *J Neurosci* 1982;2:32–48.
- Tamagnini F, Burattini C, Casoli T, Biliotti M, Fattoretti P, Aicardi G. Early impairment of long-term depression in the perirhinal cortex of a mouse model of Alzheimer's disease. *Rejuvenation Res* 2012;(in press).
- Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R. AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 2006;52:831–843.
- Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, Canossa M. Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc Natl Acad Sci USA* 2004;101:15788–15792.
- Santi S, Cappello S, Riccio M, Bergami M, Aicardi G, Schenk U, Matteoli M, Canossa M. Hippocampal neurons recycle BDNF for activity-dependent secretion and LTP maintenance. *EMBO J* 2006;25:4372–4380.
- Soulä J, Messaoudi E, Bramham CR. Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. *Biochem Soc Trans* 2006;34:600–604.
- Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Res Rev* 2008;59:201–220.
- Zeng Y, Tan M, Kohyama J, Sneddon M, Watson JB, Sun YE, Xie CW. Epigenetic enhancement of BDNF signaling rescues synaptic plasticity in aging. *J Neurosci* 2011;31:17800–17810.
- Fumagalli F, Racagni G, Riva MA. The expanding role of BDNF: A therapeutic target for Alzheimer's disease? *Pharmacogenomics J* 2006;6:8–15.
- Bertoni-Freddari C, Mocchegiani E, Malavolta M, Casoli T, Di Stefano G, Fattoretti P. Synaptic and mitochondrial physiopathological changes in the aging nervous system and the role of zinc ion homeostasis. *Mech. Ageing Dev* 2006;127:590–596.
- Reddy PH, Manczak M, Mao P, Calkins MJ, Reddy AP, Shirendeb U. Amyloid-beta and mitochondria in aging and Alzheimer's disease: implications for synaptic damage and cognitive decline. *J Alzheimers Dis* 2010;20 Suppl 2:S499–512.
- Platano D, Bertoni-Freddari C, Fattoretti P, Giorgetti B, Grossi Y, Biliotti M, Casoli T, Di Stefano G, Aicardi G. Structural synaptic remodeling in the perirhinal cortex of adult and old rats following object-recognition visual training. *Rejuvenation Res* 2006;9:102–106.
- Platano D, Fattoretti P, Biliotti M, Giorgetti B, Casoli T, Di Stefano G, Bertoni-Freddari C, Aicardi G. Synaptic remodeling in hippocampal CA1 region of aged rats correlates with better memory performance in passive avoidance test. *Rejuvenation Res* 2008;11:341–348.
- Di Stefano G, Casoli T, Platano D, Fattoretti P, Biliotti M, Giorgetti B, Bertoni-Freddari C, Lattanzio F, Aicardi G. Differences in gene expression in the hippocampus of aged rats associated with better long-term memory performance in passive avoidance test. *Rejuvenation Res* 2010;13:224–228.
- Casoli T, Di Stefano G, Fattoretti P, Giorgetti B, Biliotti M, Lattanzio F, Aicardi G, Platano D. Dynamin binding protein gene expression and memory performance in aged rats. *Neurobiol Aging* 2012;33:618.e15–618.e19.
- Biliotti M, Casoli T, Di Stefano G, Giorgetti B, Aicardi G, Fattoretti P. Ketogenic diets: An historical antiepileptic

- therapy with promising potentialities for the aging brain. *Ageing Res Rev* 2010;9:273–279.
27. Balietti M, Giorgetti B, Fattoretti P, Grossi Y, Di Stefano G, Casoli T, Platano D, Solazzi M, Orlando F, Aicardi G, Bertoni-Freddari C. Ketogenic diets cause opposing changes in synaptic morphology in CA1 hippocampus and dentate gyrus of late-adult rats. *Rejuvenation Res* 2008;11:631–640.
28. Balietti M, Fattoretti P, Giorgetti B, Casoli T, Di Stefano G, Platano D, Aicardi G, Lattanzio F, Bertoni-Freddari C. Effect of two medium chain triglycerides-supplemented diets on synaptic morphology in the cerebellar cortex of late-adult rats. *Microsc Res Tech* 2009;72:933–938.
29. Balietti M, Giorgetti B, Di Stefano G, Casoli T, Platano D, Solazzi M, Bertoni-Freddari C, Aicardi G, Lattanzio F, Fattoretti P. A ketogenic diet increases succinic dehydrogenase (SDH) activity and recovers age-related decrease in numeric density of SDH-positive mitochondria in cerebellar Purkinje cells of late-adult rats. *Micron* 2010;41:143–148.

Address correspondence to:

Giorgio Aicardi

Department of Human and General Physiology

University of Bologna

Via San Donato 19/2

40127 Bologna

Italy

E-mail: giorgio.aicardi@unibo.it