Novel Players in the Aging Synapse: Impact on Cognition

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While neuronal loss has long been considered as the main contributor to age-related cognitive decline, these alterations are currently attributed to gradual synaptic dysfunction driven by calcium dyshomeostasis and alterations in ionotropic/metabotropic receptors. Given the key role of the hippocampus in encoding, storage, and retrieval of memory, the morpho- and electrophysiological alterations that occur in the major synapse of this network—the glutamatergic—deserve special attention. We guide you through the hippocampal anatomy, circuitry, and function in physiological context and focus on alterations in neuronal morphology, calcium dynamics, and plasticity induced by aging and Alzheimer's disease (AD). We provide state-of-the-art knowledge on glutamatergic transmission and discuss implications of these novel players for intervention. A link between regular consumption of caffeine—an adenosine receptor blocker—to decreased risk of AD in humans is well established, while the mechanisms responsible have only now been uncovered. We review compelling evidence from humans and animal models that implicate adenosine A_{2A} receptors (A_{2A}R) upsurge as a crucial mediator of age-related synaptic dysfunction. The relevance of this mechanism in patients was very recently demonstrated in the form of a significant association of the A_{2A}R-encoding gene with hippocampal volume (synaptic loss) in mild cognitive impairment and AD. Novel pathways implicate A_{2A}R in the control of mGluR5-dependent NMDAR activation and subsequent Ca²⁺ dysfunction upon aging. The nature of this receptor makes it particularly suited for long-term therapies, as an alternative for regulating aberrant mGluR5/NMDAR signaling in aging and disease, without disrupting their crucial constitutive activity.

Keywords: aging, adenosine A_{2A} receptor, synaptic plasticity, hippocampus, memory, NMDA receptor, mGluR5 receptor

Introduction

AGING HAS BEEN widely associated with cognitive decline and synaptic dysfunction and is the main risk factor for Alzheimer's disease (AD). One brain region that is clearly crucial for normal episodic memory is the hippocampus. This medial temporal lobe (MTL) structure has been shown to undergo functional changes over the lifespan. During normal aging, the numbers of primary hippocampal glutamatergic neurons counted stereologically in humans, monkey, or rats do not change significantly (see Morrison and Hof¹). If cell numbers are not affected, memory impairment is probably due to the subtle changes known to occur at the synapse that result in altered mechanisms of plasticity.

The Hippocampus

The hippocampus is a three-layered structure that is mutually connected to other cortical and subcortical areas (Fig. 1A, B). The trisynaptic pathway from the dentate gyrus (DG) to the CA3 via mossy fibers and onward to CA1 via Schaffer collaterals is the principal feed-forward circuit involved in the process of information through the hippocampus² (Fig. 1C, D). The hippocampus receives unidirectional input from the entorhinal cortex (EC), where layer II neurons project to DG granule cells via the perforant path^{2,3} and layer III neurons project to CA1 neurons via the temporoammonic path (perforant path to CA1).² CA1 pyramidal cells—the major output neurons—project back to deep layers of the EC and to various subcortical and cortical areas via the subicular complex.²

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FIG. 1. Hippocampal anatomy and circuitry. (A) Principal anatomy of the human hippocampal memory systems and the brain regions involved in learning and memory. (B) Schematic rat brain with the hippocampal formation highlighted. (C) Schematic hippocampal slice. (D) Hippocampal slice with different areas and layers. (E) Schematic representation of the hippocampal trisynaptic circuit. First, granule neurons in the hippocampal dentate gyrus receive afferent inputs, via the perforant path, from the layer II of the lateral and MEC. Next, granule neurons project to the CA3 pyramidal neurons via mossy fibers and, ultimately, CA1 neurons receive inputs from the CA3 by the Schaffer collaterals, by the contralateral hippocampus through associational/commissural fibers or direct inputs from the perforant path. To close the hippocampal synaptic loop, CA1 pyramidal neurons project back to the EC. Ant, anterior thalamic nuclei; CA, *cornu ammonis*; CM, *corpus mammillaris*; DG, dentate gyrus; EC, entorhinal cortex; LEC, lateral entorhinal cortex; LPP, lateral perforant pathway; MEC, medial entorhinal cortex; Med, medial thalamic nuclei; MPP, medial perforant pathway; Mtt, mamillothalamic tract; SN, septal nucleus. Adapted from Lavenex and Amaral.⁷ Color images are available online.

The DG has three distinct layers (molecular, granular, and polymorphic) and mainly consists of granule cells. The axons of the DG granule cells form the mossy fiber system and project both to the CA3 and back onto granule cells, thus forming a recurrent network.^{2,4} In addition, the DG receives inputs from the contralateral hippocampus via commissural projections.^{2,4} Axon collaterals of CA3 pyramidal neurons synapse onto other CA3 neurons, forming a recurrent autoassociative network, whereas CA3 neurons projecting back to the dentate network form a heteroassociative network.^{2,5} CA1 pyramidal neurons not only receive information which has been preprocessed in the subnetworks of the DG

and CA3 but also receive direct projections from the EC, suggesting that the function of the CA1 neurons includes comparing new information from the EC with stored information via CA3 relevant in detection of error, mismatch, and novelty^{2,6} (Fig. 1E). The crucial role of this redundant feed-forward circuit is crucial for learning and memory and may also underlie its high vulnerability to insults^{2,7} (Fig. 1E).

Synaptic organization

The integrative properties of neurons depend strongly on the number, proportions, and distribution of excitatory

and inhibitory inputs they receive. A single CA1 pyramidal cell has ~12,000 μm dendrites and receives around 30,000 excitatory and 1,700 inhibitory inputs, of which 40% are concentrated in the perisomatic region and 20% on dendrites in the *stratum lacunosum-moleculare*.⁸ The density of dendritic spines and synapses on CA1 pyramidal neurons is highest in the *stratum radiatum* and *stratum oriens*.⁹

Hippocampal neurons mainly release glutamate or gamma-aminobutyric acid (GABA).⁹ These neuron types are, most of the times, easily identified due to significant differences in (a)symmetry of synapses, excitatory or inhibitory effect, relative somatic abundance within an area, presence of dendritic spines, and local or projecting nature of the axons.¹⁰

The cell bodies of glutamatergic pyramidal neurons are organized in a three-to-five cell-deep laminar arrangement in *stratum pyramidale* and have orthogonal dendrites from *stratum oriens* to *stratum lacunosum moleculare*, thus receiving afferent inputs from several intrinsic and extrinsic sources across well-defined dendritic domains.¹¹ In contrast, inhibitory interneurons (approximately 10–15% of the total cell population), which release the neurotransmitter GABA, have their cell bodies distributed throughout all major *strata* but they integrate from a more restricted intrinsic and extrinsic afferent input repertoire. However, some interneurons possess axons that cross considerable distances to innervate distinct subcellular compartments or alternatively form long-range projections that extend beyond their original central location to ramify within both cortical and subcortical structures.¹¹

Hippocampal interneurons can be divided into several types: neurogliaform family (32.2%), SOM expressing (9.3%), PV expressing (23.9%), CCK expressing (13.9%), and interneuron-specific (19.4%).¹¹ Despite being the minority, this diverse neuronal population serves as a major determinant of virtually all aspects of neocortical circuit function and regulation.¹¹

The hippocampus receives multiple direct and indirect projections that are crucial to hippocampal function regulation. Dopaminergic projections from both the substantia nigra *pars compacta* and the ventral tegmental area are important for memory processing.^{12,13} Also, activation of cholinergic projections activation from the medial septum/diagonal band is sufficient to induce 40-Hz network oscillations in the hippocampus *in vitro*, thus playing an important role in hippocampal memory processing.¹⁴ Serotonergic projections from the median raphe nucleus to the ventral hippocampus have also been consistently described¹⁵ and are involved in the retrieval of fear memories.¹⁶

Glutamatergic synapses

Electron microscopy images reveal structural differences between the glutamatergic pre- and postsynaptic

components (Fig. 2A). The presynaptic element is easily identified by the presence of neurotransmitter-containing vesicles, generally of relatively uniform size. These vesicles aggregate near a membrane specialization, identified by a more electrodense thickening, reflecting the presence of membrane proteins necessary for exocytosis—“active zone” (Fig. 2A). Among these are proteins that interact with vesicular partners (SNARE proteins), as well as voltage-dependent channels that mediate the influx of Ca^{2+} , which ultimately triggers exocytosis upon an action potential. In addition, mitochondria are frequently present due to the local high energetic demands coupled to transmitter release (transmitter synthesis, vesicular packaging, exocytosis, and reuptake).^{9,17}

On the postsynaptic side, the membrane is more electrodense than the presynaptic membrane (Fig. 2A). This postsynaptic density, or PSD, is a relatively detergent-resistant structure, 50 nm thick, that scaffolds as many as 100 different proteins, among them the glutamate receptors.^{9,18,19} In inhibitory neurons gephyrin is the equivalent postsynaptic scaffolding molecule.²⁰

Glutamate receptors. Glutamate is packaged into synaptic vesicles in the presynaptic terminals, and released into the synaptic cleft through the docking of synaptic vesicles to the membrane at the active zone. Glutamate then activates postsynaptic glutamate receptors to regulate several neuronal functions, from neuronal migration to excitability and plasticity.¹⁸ Glutamate receptors are highly complex transmembrane proteins that can be divided into two main categories: voltage-sensitive ionotropic glutamate receptors (iGluRs; glutamate-gated ion channels) and ligand-sensitive metabotropic glutamate receptors (mGluRs; glutamate-activated G protein-coupled receptors [GPCRs]) (Fig. 2B).

iGluRs mediate the fast excitatory transmission, acting as cation channels that open upon glutamate binding. AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are the first iGluRs activated, leading to sodium influx and consequently postsynaptic membrane depolarization (Fig. 2B–D). Following the initial AMPAR-mediated depolarization, NMDA (N-methyl-D-aspartate) receptors become activated due to removal of the voltage-dependent physical occlusion by magnesium of the channel pore and are permeable to sodium and calcium ions (Fig. 2B–D).¹⁸ Kainate receptors also mediate synaptic transmission, at a smaller extent, through the entry of sodium and calcium (Fig. 2B–D). Generally, AMPA receptors mediate fast (<10 mseconds) synaptic transmission, while NMDA and kainate receptors mediate slow (10–100 mseconds) synaptic transmission.¹⁸

In addition, mGluRs activation also modulates neuronal excitability and synaptic transmission. mGluRs are slower players since they exert their effects through recruitment of second messenger systems, gene expression, and protein synthesis. Eight mGluR subtypes (mGluR1–8)

FIG. 2. Glutamatergic pre- and postsynaptic neurons are very distinct structurally and functionally. (A) Electron micrographs of the CA1 area of the hippocampus showing morphological differences between pre- and postsynaptic components. The presynaptic element is easily identified by the presence of neurotransmitter-containing vesicles, generally of relatively uniform size. These vesicles aggregate near a membrane specialization that can be identified as a thickening, reflecting the presence of membrane proteins necessary for exocytosis—"active zone." On the postsynaptic side, there is also an increased density of the membrane—postsynaptic density, a relatively detergent-resistant structure containing glutamate receptors and associated macromolecules (image kindly provided by Andreia Pinto, IMM JLA). (B) Most abundant subunit composition of ionotropic receptors AMPA, NMDA, and kainate in the hippocampus.¹⁸ (C) Representative AMPAR and NMDAR excitatory postsynaptic currents measured in a rat CA1 hippocampal neuron at -70 and +40 mV.¹⁰⁰ (D) Schematic diagram of a glutamatergic synapse. Calcium influx through activation of pre-synaptic VDCC drives docking of the glutamate vesicles to the membrane. Once released, glutamate acts on postsynaptic AMPA (sodium influx), NMDA (sodium and calcium influx), kainate (sodium influx), and metabotropic receptors; adapted from Hassel and Dingledine.¹⁸ AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluA, AMPA receptor subunit; GluK, kainate receptor subunit; GluN, NMDA receptor subunit; NMDA, N-methyl-D-aspartate; VDCC, voltage-dependent calcium channels. Color images are available online.

are differentially expressed in specific regions in the central nervous system (CNS), being divided into three subgroups based on sequence homology, G protein-coupling, and ligand selectivity (Fig. 2D).¹⁸ Group I mGluRs (mGluR1 and 5) are widely expressed postsynaptically, being preferentially associated with Gq/Gs.¹⁸

Group I mGluRs trigger the activation of phospholipase C (PLC), which then leads to calcium mobilization from endoplasmic reticulum and protein kinase C (PKC) activation.¹⁸ Glutamatergic soma and synapses can be labeled by a wide variety of markers listed in Table 1.

TABLE 1. GLUTAMATERGIC SYNAPTIC MARKERS, LOCALIZATION, AND ROLE

Marker	Localization	Role
AMPA GluA1	PSD	AMPA receptor R1 subunit
CaMKII	Neuronal soma	Protein kinase regulated by the Ca^{2+} /calmodulin complex; involved in Ca^{2+} homeostasis and in learning and memory processes ³²
Glutaminase	Neuronal soma	Enzyme that generates glutamate from glutamine ¹⁸
Glutamine synthase	Astrocytes	Enzyme that catalyzes the condensation of ammonia and glutamate to form glutamine ¹⁸
NMDAR	PSD	NMDA receptor N1 subunit
GluN1	PSD	Fibrous specialization of the submembrane cytoskeleton that adheres to the postsynaptic membrane; regulation of adhesion, control of receptor clustering, and regulation of receptor function ^{9,18,19}
PSD95	PSD	
vGluT	Pre-synaptic neuron	Transports cytoplasmic glutamate into vesicles ³⁵

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaMKII, Ca^{2+} /calmodulin-dependent protein kinase II; NMDA, N-methyl-D-aspartate; PSD, postsynaptic density; vGluT, glutamate vesicular transporter.

Synaptic plasticity

Synaptic plasticity can be defined as activity-dependent modifications in the efficacy and strength of synaptic transmission of preexisting synapses.²¹ Long-term synaptic plasticity can last from minutes to several days and even years.^{22–24} These synaptic plasticity processes have long been correlated with memory performance and are proposed to be a main neurophysiological correlate of memory.^{21,25}

Hebbian plasticity. The Schaffer collaterals-CA1 synapse is undoubtedly the most well-characterized and well-studied glutamatergic synapse of the hippocampus. In the last decades, many *in vitro* electrophysiological studies have described the events underlying long-term potentiation (LTP) and long-term depression (LTD) phenomena. These mechanisms, found and studied in animal models, have also been described in humans.²⁶ Importantly, blockade of proteins involved in either LTP or LTD mechanisms disrupts learning.^{27–30}

The most extensively studied and characterized forms of synaptic plasticity are the LTP and LTD in the Schaffer collaterals-CA1 region of the hippocampus. LTP is defined as the long-lasting enhancement in synaptic transmission between two neurons following a continuous and strong stimulation (high-frequency stimulation or theta-burst stimulation), while LTD reflects a long-lasting decrease in the efficiency of synaptic transmission following a continuous weak stimulation (LFS: low-frequency stimulation).

Postsynaptically, LTP and LTD processes begin with a depolarization of the membrane due to Na^+ influx through AMPAR activation, which releases the Mg^{2+} block from NMDAR. Although both forms require AMPAR and NMDAR activation, it is the spatiotemporal nature of the intracellular Ca^{2+} rise that dramatically impacts the direction of plasticity.³¹ The huge increase in Ca^{2+} influx elicited by strong activation of NMDAR acti-

vates calcium/calmodulin-dependent protein kinase II (CaMKII), a key component of the molecular machinery for LTP, since LTP induction was prevented in knockout (KO) mice lacking a critical CaMKII subunit.³² The modest increase in Ca^{2+} influx observed upon weak stimulation instead drives activation of phosphatases, such as protein phosphatase 1 and 2.³³ Thus, the probability of a given synapse to undergo LTP or LTD is a function of kinases versus phosphatases activation. Consequently, in LTP or LTD AMPAR phosphorylation can be regulated bidirectionally, with LTP increasing phosphorylation and LTD decreasing phosphorylation.^{34–37}

Homeostatic plasticity. Most studies of long-term changes in synaptic strength have focused on Hebbian mechanisms, where these changes occur in a synapse-specific manner. Although Hebbian mechanisms are necessary for modulating neuronal circuitry selectively, they might not be sufficient since they tend to destabilize the activity of neuronal networks. An increase in synaptic strengths, such as in LTP, would increase the excitatory drive on to a postsynaptic cell, making it more likely to fire. This in turn would increase the likelihood of more LTP; thus positive feedback would quickly saturate the system, resulting in a hyperactive state with saturated synaptic inputs. Conversely, excessive LTD would also proliferate, resulting in a silent state with inputs fully depressed.³⁸

Homeostatic plasticity acts as a compensatory stabilizing mechanism that, using a negative feedback system, keeps the activity of the network within a dynamic range.^{39,40} Several forms of homeostatic plasticity have been identified and include mechanisms that regulate neuronal excitability, stabilize total synaptic strength, and influence the rate and extent of synapse formation.⁴¹ These forms of homeostatic plasticity are likely to complement Hebbian mechanisms to allow the modification of neuronal networks selectively.⁴¹ In essence, the whole synaptic population is equally affected, such that the overall sum of synaptic strengthening and therefore activity of a neuron is changed but the relative

weighed differences between synapses is preserved: the computational and storage capacity of the network is not compromised and homeostatic plasticity will not be in conflict with or erase the information set by Hebbian plasticity.⁴¹

The Aged Glutamatergic Synapse

According to the World Health Organization (WHO), by 2020, the number of people aged 60 years and older will outnumber children younger than 5 years. Moreover, between 2015 and 2050, the proportion of the world's population over 60 years will nearly double from 12% to 22%. Aging is, in fact, the main risk factor for AD.⁴² These profound demographic changes have placed "the aging process" as one of the big challenges for scientific research nowadays. Although aging affects the entire body, its impact on brain and cognition has a profound effect on quality of life of the individuals. Much work has been focused on the hippocampus, as age-related decline in performance dependent on this region is consistently found across species and tasks.^{43–48}

These age-related memory impairments can be explained, in part, by changes in neural plasticity or cellular alterations that directly affect mechanisms of plasticity.⁴⁹ Although several age-related neurological changes have been identified during normal aging, these tend to be subtle compared with the ones observed in age-associated disorders, such as AD and Parkinson's disease (PD).⁴⁹ Consequently, understanding age-related changes in cognition sets a background against which it is possible to assess the effects of the disease.⁴⁹

For a long time, aging had been associated with neuronal loss independently of the brain region.^{50–53} However, the methods used in those studies question the accuracy of such results^{1,49} and subsequent studies have conclusively shown that the cell number is preserved in aging in several brain areas, including the hippocampus.^{54–59} These data highlight the differences between normal aging, characterized by structural preservation in the MTL, versus AD, which is associated with neuronal and synaptic loss in the MTL and in the hippocampus in particular.⁶⁰

Although the total number of Schaffer collaterals-CA1 synapses is preserved across different age groups,⁶¹ the amplitude of the Schaffer collaterals-induced field excitatory postsynaptic potentials (fEPSP) recorded in CA1 is reduced in aged memory-impaired animals.^{62–64} Furthermore, the PSD area of axospinous synapses is significantly reduced in aged learning-impaired rats⁶⁵ (Fig. 3A). However, at the CA3-CA1 synapse, the size of the unitary EPSP remains constant during aging⁶⁶ (Fig. 3A). Together, these data suggest that aging might not be associated with alterations in the strength of individual synaptic connections but instead with an increase in nonfunctional or silent synapses in the hippocampus^{49,67} (Fig. 3A).

Recently, transcriptomic analysis of the human aged brain revealed robust negative associations of genes encoding pre- and postsynaptic proteins with age, likely related to functional changes in synaptic integrity seen with aging (for a complete review see Burke and Barnes^{49,67}). Interestingly, these changes occur across inhibitory and excitatory synapses and some of the strongest effects were observed in the hippocampus, consistent with the increased vulnerability of this structure to the aging process.⁶⁸

Alterations in glutamate metabolism have also been described upon aging. In the hippocampus of mice and rats, the glutamate content in tissue samples decreases at 24–29 months,^{69–71} which may explain age-related changes in hippocampal neuron function during aging. However, basal extracellular concentrations of glutamate in aged rodents were reported to be either greater or lower, providing contradictory results,^{71,72} and glutamate uptake, which terminates its action in the synapse, does not seem to be altered in aging.^{73–75} These results are difficult to reconcile, but this may be due to the fact that the concentration of glutamate sampled in most of these studies results from the release and uptake processes. Also, since most of these techniques cannot differentiate between neuronal and glial sources, the cellular origin of the glutamate released is still uncertain.⁷⁶ In addition, age-related decreases in presynaptic release of glutamate may be compensated by changes in uptake and may also reflect alterations in the density of postsynaptic glutamate receptors.

In rodents, one of the well-characterized markers of physiological aging is an age-related decrease in action potential firing rates of CA1 pyramidal cells, with a concomitant decrease in the amplitude of the postburst after hyperpolarization responsible for spike frequency adaptation,⁷⁷ reviewed by Wu *et al.*⁷⁸ Besides intrinsic properties, the excitatory synaptic transmission is altered during aging, being mainly studied in the hippocampus of old rats, and/or cortex of primates.⁷⁸

Such alterations observed at individual synapses have a significant impact on synaptic plasticity. Age-associated memory deficits correlate with impairments in either LTP or LTD.^{28,79} In aged animals, LTP has been found to be reduced^{43,80–82} (Fig. 3B), not altered^{81,83–87} (Fig. 3B) or even strengthened.^{83,88–91} The latter is inconsistent with the classical correlation between increased LTP magnitude and better performance on hippocampal-dependent memory tasks. Differences in the synaptic circuit that is being potentiated⁸¹ or in the stimulation protocol^{49,83} may account for the observed discrepancies in LTP magnitude. Generally, age-associated alterations in LTP are only observed when weaker stimulus protocols are used, resulting in either an increase^{83,88,89,91} or in a decrease⁷⁹ of LTP (Fig. 3B).

Given the key role of AMPAR in LTP and LTD, synaptic plasticity alterations observed upon aging may be

FIG. 3. Morphological and synaptic alterations upon aging in the CA1 area of the hippocampus. (A) Morphological and electrophysiological impairments observed upon aging in CA1; increased VDAC and aberrant NMDA receptor function impair calcium homeostasis, further enhanced by a decrease in activity of calcium buffering proteins, driving alterations in gene regulation. (B) Examples of LTP and LTD time courses upon different stimulation protocols (TBS: 10 trains with 4 pulses at 100 Hz, separated by 200 mseconds; HFS: 4 trains of 100 pulses at 100 Hz, separated by 5 minutes; low-frequency stimulation: 3 trains of 1200 pulses at 2 Hz, separated by 10 minutes); representative traces of fEPSPs before (*black*) and 50–60 minutes after (*gray, green*) LTD or LTP induction in young and aged rats.¹⁰⁰ * $p < 0.05$. CBPs, calcium-binding proteins; fEPSPs, field excitatory postsynaptic potentials; HFS, high-frequency stimulation; LFS, low-frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; TBS, theta-burst stimulation. Color images are available online.

related with alterations in AMPAR expression and function, although very few studies have addressed that. Administration of AMPAR positive allosteric modulators restores age-related memory and synaptic potentiation deficits,^{92,93} suggesting an increase in silent

AMPA rather than alterations in the expression of synaptic AMPAR.⁹⁴

The transcription factor cAMP response element-binding protein (CREB) has been shown to have fundamental roles in cognition and cellular excitability.^{95,96}

FIG. 4. Adenosine A_{2A}R, distributed heterogeneously throughout the body, have important physiological functions in neurons, astrocytes, and microglia. **(A)** A_{2A}R are particularly expressed in the lungs, spleen, thymus, heart, blood vessels, muscle, and brain.²⁶⁷ **(B)** A_{2A}R are highly expressed in the olfactory bulb and striatum, whereas in the neocortex and hippocampus, they are present at residual levels. **(C)** Exogenous A_{2A}R activation induces a presynaptic enhancement of phasic GABAergic inputs from parvalbumin-expressing neurons to other GABAergic INs, driving disinhibition of PYR (green line), whereas A_{2A}R activation in PYR, presynaptically located, enhances glutamatergic inputs to other glutamatergic neurons (green line); A_{2A}R do not affect neither monosynaptic inhibitory inputs to excitatory neurons nor monosynaptic glutamatergic inputs to INs (black line). **(D)** A_{2A}R, predominantly presynaptic, increase the release of glutamate in the hippocampus, possibly by inducing Ca²⁺ uptake and PKA-dependent Ca²⁺ currents through P-type Ca²⁺ channels in the presynaptic CA3 neurons that project onto the CA1 PYR. Postsynaptically, A_{2A}R facilitates AMPAR-evoked currents via PKA and increases mGluR5-dependent NMDAR phosphorylation and NMDAR-responses in CA1. However, pre- or postsynaptic localization of A_{2A}R and whether this is a direct or indirect interaction is lacking. In astrocytes, A_{2A}R activation induces astrocytic proliferation and activation and decreases glutamate uptake by controlling the expression levels of glutamate transporters subtypes GLT-1 and GLAST.^{220,279,280} Microglial A_{2A}R drive proliferation and activation, process retraction and release of important immune mediators, particularly cyclooxygenase, PGE2, NO, and IL-1 β .^{281–286} A_{2A}R, A_{2A} receptors; CA1 and CA3, *cornu ammonis* 1 and 3; CCK+, cholecystikinin-positive interneuron; COX2, cyclooxygenase 2; GABA, gamma-aminobutyric acid; IL-1 β , interleukin-1 β ; IN, interneuron; NO, nitric oxide; PGE2, prostaglandin E2; PV+, parvalbumin-positive interneuron; PYR, pyramidal cell; SLM, *stratum lacunosum-moleculare*; SO, *stratum oriens*; SP, *stratum pyramidale*; SR, *stratum radiatum*. Color images are available online.

The first study that identified an age-related CREB signaling dysfunction showed a reduction in LTP and performance in Barnes test that was ameliorated upon treatment with compounds that activate the cAMP/PKA pathway, possibly by increasing CREB activity.⁹⁷ Levels of phosphorylated CREB (pCREB) are decreased in aged versus young animals after training in the Morris water maze, and strongly correlated with individual learning performance, whereas CREB expression itself does not change.⁹⁸ These results suggest that alterations in activation rather than expression contribute to the age-related changes in cognition.

Some authors report increased susceptibility to LTD during aging,⁸⁷ whereas others fail to observe alterations in LTD magnitude in aged animals.^{84,99} These discrepancies can be explained by differences in animal strain, stimulation pattern, or $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio. Accordingly, paired-pulse LFS (PP-LFS) does not induce changes in LTD between young and aged animals,⁹⁹ suggesting that different mechanisms may be involved in the induction of LTD by LFS and PP-LFS. Also, age-related differences in LTD induction could be rescued by manipulating the extracellular $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio. Indeed, the fact that induction of LTD is both a function of age and the levels of Ca^{2+} in the recording medium, strongly support an age-related Ca^{2+} dysregulation, and a shift in Ca^{2+} -dependent induction mechanisms rather than in the LTD intrinsic capacity.^{84,99} Data produced by our group are consistent with this hypothesis, since we showed that aging is associated with a shift in the form of plasticity induced by a weak stimulus (LFS elicited LTP instead of LTD), as a consequence of increased NMDAR activation and Ca^{2+} influx¹⁰⁰ (Fig. 3B).

It has been hypothesized that postsynaptic intracellular levels of Ca^{2+} are involved in setting a synaptic modification curve, which determines the probability that a synapse will be depressed or potentiated for a given pattern of input.^{61,101} Accordingly, since Ca^{2+} homeostasis is disrupted in aged animals (discussed further below),^{102,103} we can expect alterations in the probability for a given synapse to undergo potentiation or depression. All these observations support the *calcium hypothesis* of aging, which implicates raised intracellular Ca^{2+} as the major source of functional impairment and degeneration in aged neurons.^{104–106}

To avoid excessive intracellular levels of calcium ($[\text{Ca}^{2+}]_i$) elevations, neurons are equipped with complex machinery that permanently modulates the temporal and spatial patterns of Ca^{2+} signaling.^{107,108} Brief elevations of $[\text{Ca}^{2+}]_i$ are essential in controlling membrane excitability and modulating synaptic plasticity mechanisms, gene transcription, and other major cellular functions.^{107,108} However, long-lasting elevation of $[\text{Ca}^{2+}]_i$ triggers neurotoxic signaling pathways that ultimately will drive cell death.^{107,108}

Several studies reported an age-associated increase in basal $[\text{Ca}^{2+}]_i$ levels^{109,110} as a result of increased voltage-

dependent Ca^{2+} influx,^{103,111,112} aberrant buffering^{113–115} (Fig. 3A), extrusion,^{110,116–118} and uptake capacity.¹¹⁹ Furthermore, given the key role of NMDAR in synaptic plasticity and memory,¹²⁰ putative alterations in NMDAR may also account for Ca^{2+} dysregulation.

In aged CA1 pyramidal neurons, there is an increased duration of NMDAR-mediated responses.¹²¹ Consistent with this hypothesis, aged animals display an NMDAR overactivation upon glutamate or glycine stimulation, despite a decrease in the density of these receptors¹²² (Fig. 3A). However, such alteration in NMDAR-mediated responses may be due to the previously described increase in nonfunctional synapses with aging. The fact that there is an increased binding of the NMDAR antagonist MK-801 in animals with learning and retention deficits^{123,124} suggest that an increase in NMDAR channel open-time happens as a compensatory mechanism for the apparent decrease in receptor number,¹²⁵ since MK-801 only binds open channels.

The age-associated synaptic dysfunction can also be a consequence of alterations in astrocytes and microglia, as the aging process has also been described as *inflammaging*, a status of chronic inflammation that contributes to the pathogenesis of neurodegenerative diseases.¹²⁶ Although the number of astrocytes remains unaffected^{127–129} in aged humans, in rats there seems to be an increase in the astrocytic size, described in the hippocampus.¹²⁹ In mice, age decreases the expression of ionotropic and purinergic receptors¹³⁰ and neurotransmitter-induced Ca^{2+} signaling.¹³¹ Importantly, age is also associated with reduced expression of water channels (aquaporins 4) in astroglial perivascular processes and markedly diminished clearance of the brain parenchyma through the glymphatic pathway,¹³² a key process in the prevention of the accumulation of misfolded protein aggregates.

Microglia alterations upon aging have also been described in several species. Age-dependent microglia activation was found in aged rodents, nonhuman primates, and humans,^{133–135} characterized by increased expression of MHCII, CD68, TLRs, and proinflammatory cytokines such as TNF α , interleukin-1 β (IL-1 β), and IL-6.^{136–140} However, other studies unravel a microglial dystrophic/senescent phenotype in aged individuals,^{141,142} thus supporting the hypothesis that, rather than induction of microglial activation, progressive microglial degeneration and loss of microglial neuroprotection are associated with aging and further contribute to the onset and progression of neurodegenerative diseases such as AD.

The Modulation of Aged Synapse by Adenosine A_{2A} Receptors

Adenosine influences many functions in the CNS. Besides the neuromodulatory actions, adenosine acts as a fine-tuner of synaptic communication, as it is a relevant player in neuron–glia communication and can affect the

release and action of many neurotransmitters and other neuromodulators.^{143–147} Accordingly, the neuromodulatory role of adenosine is mediated by a balance between the inhibitory and excitatory actions via A₁R (usually coupled to adenylate cyclase inhibitory proteins, Gi/Go) and A_{2A} receptors (A_{2A}R; coupled to adenylate cyclase inhibitory proteins Gs), the most relevant adenosine receptors in the CNS.¹⁴⁸ The effects of adenosine depend on the receptors expression pattern and signaling, the brain region, and pathophysiological condition. A₁R are widely distributed, being more abundant in the cortex, cerebellum, and hippocampus.¹⁴⁹ On the opposite, A_{2A}R display a more restricted expression pattern: A_{2A}R are highly expressed in the olfactory bulb and striatum,¹⁵⁰ whereas in the neocortex and hippocampus they are present at residual levels^{151,152} (Fig. 4A, B). A_{2A}R are mostly located in glutamatergic synapses,¹⁵³ although they have been shown in other synapses, such as GABAergic^{146,154,155} (Fig. 4C), dopaminergic,^{156,157} cholinergic,^{158,159} serotonergic,^{160,161} or noradrenergic synapses.¹⁶²

Adenosine levels are tightly controlled by a complex machinery of enzymes. However, in the aged rat, the activity of the enzymes that form adenosine from ATP (5'-nucleotidases) and terminate adenosine actions by phosphorylation to AMP (adenosine kinase, ADK) have been reported to be increased, possibly leading to the reported elevation of adenosine levels in the brain^{163–165} and interference with the transmethylation pathway. This can at least explain the decrease in global DNA methylation observed upon aging.

Glucose metabolism impairment or mitochondria dysfunction, common features observed in aging, and AD may lead to the reduction of cellular ATP levels and trigger the process of ATP production from adenosine. Accordingly, ATP levels are significantly reduced in the brain of APPswe/PS1dE9 mice¹⁶⁶ and can lead to a reduction in adenosine levels and mitochondria dysfunction.^{167–169} Adenosine acts as a cellular sensor of intracellular ATP levels: elevation of adenosine in response to falling ATP levels has cytoprotective effects, whereas when decoupled from ATP levels can be cytotoxic to the cell.¹⁶⁸ In AD, adenosine levels have been categorized according to AD stage.¹⁶⁷ Alonso-Andrés *et al.* showed that adenosine is the most affected purine in AD, being decreased since early stage,¹⁶⁷ possibly as a result of a decrease in 5'-nucleotidases.¹⁷⁰ Adenosine augmentation has been found beneficial in multiple neurological disorders, including AD.^{171,172} However, further comprehension of the mechanism and the putative effects on adenosine receptors and downstream signaling pathways are crucial at this stage.

In glutamatergic synapses, under physiological conditions and basal activity, adenosine preferentially stimulates A₁R, leading to inhibition of glutamatergic synaptic transmission in the hippocampus.^{173,174} Adenosine can also activate A_{2A}R, which decreases A₁R binding and

thus an inhibition of A₁R actions.¹⁴³ Several studies addressed the cellular mechanisms triggered by A_{2A}R activation, either by modulating the adenosine endogenous levels pharmacologically and/or by applying its specific agonist, such as CGS21680, allowing a comprehensive knowledge of its action, as follows. A_{2A}R, predominantly presynaptic,¹⁵³ increase the release of glutamate in the hippocampus,^{143,175} possibly by inducing Ca²⁺ entry¹⁷⁶ and PKA-dependent Ca²⁺ currents through P-type Ca²⁺ channels in the presynaptic CA3 neurons¹⁷⁷ that project onto the CA1 pyramidal cells (Fig. 4C, D). Postsynaptically, A_{2A}R facilitates AMPAR-evoked currents via PKA in CA1 pyramidal neurons, independent of NMDAR and GABA_A receptor activation or synaptic activity¹⁷⁸ (Fig. 4D). In mossy fiber synapses, A_{2A}R activation along the extrasynaptic plasma membrane of CA3 dendritic spines is essential for LTP of NMDAR-EPSCs.¹⁷⁹ This LTP is dependent on postsynaptic Ca²⁺ rise, NMDAR, mGluR5, and Src tyrosine kinases family activation.¹⁷⁹ This was the first article that provided electrophysiological evidence and biological relevance for a A_{2A}R-mGluR5-NMDAR interaction.

Other studies also hinted at a possible A_{2A}R-NMDAR interaction, since A_{2A}R activation increases mGluR5-dependent NMDAR phosphorylation and NMDAR-responses in CA1.^{180–182} However, the exact pre- or postsynaptic localization of A_{2A}R and whether this is a direct or indirect interaction is yet to be clarified (Fig. 4D). Also, A_{2A}R seem to act as fine-tuners of other neuromodulatory systems, since A_{2A}R activation is required to observe synaptic effects of neuropeptides^{145,183–185} or growth factors, namely for the facilitatory actions of brain-derived neurotrophic factor (BDNF) on synaptic transmission^{145,185,186} and on LTP.¹⁸⁷ Furthermore, A_{2A}R activation decreases the efficiency of presynaptic inhibitory systems, namely cannabinoid CB1 receptors.^{188,189} However, whether these effects are mediated by neuronal or glial A_{2A}R is still a matter of debate. As indicated, some of the above mentioned effects of A_{2A}R on glutamate transmission were observed upon exogenous activation with agonists, such as CGS 21630.^{143,175,176,178} However, this may not replicate exactly the endogenous activation-mediated actions.

The comprehensive characterization of the full A_{2A}R KO mice allowed to study the role of these receptors under physiological conditions. These mice display reduced exploratory activity, increased anxiety, and aggressiveness.¹⁹⁰ Furthermore, mice lacking A_{2A}R show improved spatial recognition memory¹⁹¹ and preferential enhancement of working memory.¹⁹²

However, chronic or acute blockade of A_{2A}R does not alter neither basal glutamate transmission nor the magnitude of LTP or LTD,^{100,193,194} and knocking-out the A_{2A}R does not impact on LTD,¹⁹⁵ strongly suggesting a lack of A_{2A}R constitutive activation in young glutamatergic synapses. This idea is further supported by the

absence of effect of A_{2A}R chronic blockade or genetic deletion in the Morris water maze and Y-maze tests.^{100,193,195,196} A question might be raised: How could A_{2A}R behavioral phenotypes, which suggest some memory processes, are upon the control of A_{2A}R, be reconciled with the absence of an A_{2A}R constitutive activation in young glutamatergic synapses? Novel mice models with neuronal circuitry/cell type selective A_{2A}R deletion will allow to dissect which synapses/cells are implicated in the mechanisms underlying these memory phenotypes, excluding artefacts/compensations due to full genetic ablation of the receptor.

A_{2A}R expression and signaling is profoundly altered in the hippocampus upon aging. A_{2A}R density and coupling to G protein is increased,^{100,144,164,197–199} probably enhancing the action of this receptor to facilitate neurotransmitters release in glutamatergic synapses by a presynaptic mechanism.¹⁹⁷ However, whether this increase in G protein coupling is due to receptors covalent modifications or other alterations is not known yet.^{144,197} This age-related enhanced A_{2A}R-mediated facilitation of synaptic transmission is dependent on PKA and is associated to an increase in cAMP accumulation.¹⁴⁴ Other authors also observed an enhanced role of A_{2A}R in the modulation of LTP, since A_{2A}R antagonist SCH58261-induced decrease in LTP is increased in aged animals,¹⁹⁹ either due to alterations in A_{2A}R or changes in dynamic range of LTP.

Although at physiological levels, the expression of A_{2A}R in the hippocampus seems to be protective, by

fine-tuning the function of other protein-partners, such as the BDNF-TrkB signaling,^{145,185–187} the fact is that A_{2A}R overexpression, in a similar magnitude, to the one observed in human aging, is deleterious, as it is sufficient to trigger synaptic and cognitive deficits. This effect involves a mGluR5-dependent NMDAR overactivation, leading to enhanced Ca²⁺ influx,¹⁰⁰ which recapitulates the main synaptic alterations observed upon aging (see previous section). The way mGluR5 activates NMDAR is still unclear, and several alternatives are plausible. mGluR5 are linked to NMDAR via a Homer/Shank/PSD-95 complex of proteins^{200–202} and several studies have found that mGluR5 enhances NMDAR currents through a PKC/IP₃-calcium-dependent mechanism.^{203–206} Our group provided evidence that A_{2A}R activation mediates mGluR5-dependent NMDAR phosphorylation of the residue Tyr1472 of GluN2B subunit by Fyn kinases activation in a PD model¹⁹³ (Fig. 5), as previously reported by others in physiological conditions.¹⁸¹

Whether mGluR5 activates NMDAR by multiple pathways in the same synapses or if each pathway is region-specific is still unclear. Another important target of the kinase activity of Fyn is tau, and this interaction was already shown to be important in the AD-related tau hyperphosphorylation^{207,208} (Fig. 5). Physiologically, Fyn regulates NMDAR activity.^{209,210} On the contrary, tau-Fyn interaction leads to Fyn accumulation in the soma, preventing Fyn from migrating to postsynaptic site,²⁰⁸ possibly leading to aberrant NMDAR signaling observed in aging and AD.

FIG. 5. The aging synapse: in the CA1 area of the hippocampus, A_{2A}R increased levels are associated with postsynaptic impairments, such as a mGluR5-dependent NMDAR activation, possibly through Fyn Kinases. NMDAR aberrant activation and VDCC increased expression trigger an increase in Ca²⁺ influx. Associated with a dysfunction of CBPs, this mechanism shifts Ca²⁺-dependent induction mechanism, which explains the alterations observed in LTP and LTD upon aging. Ca²⁺ dyshomeostasis and the associated synaptic plasticity shift impair gene regulation, further exacerbating synaptic dysfunction. Color images are available online.

One of the hypothetical mechanisms for these age-associated alterations in A_{2A}R is a shift in dimerization process. In physiological conditions, A₁R and A_{2A}R are coexpressed in CA1 pyramidal neurons¹⁵³ and A_{2A}R activation decreases presynaptic A₁R binding and functional responses in aged animals via PKC.²¹¹ Importantly, attenuation of A₁R responses elicited by A_{2A}R activation is larger in amplitude than the direct facilitatory effect of A_{2A}R agonist CGS21680, suggesting that the main role of A_{2A}R in young animals is to modulate A₁R responses rather than to directly facilitate neuronal excitability.²¹¹ These age-associated A₁R-A_{2A}R cross talk loss and amplification of A_{2A}R-mediated responses suggest alterations in the organization and relative densities of GPCR and intracellular pathways, namely change in nature of the interaction, that is, homodimer A_{2A}R-A_{2A}R rather than heterodimer A_{2A}R-A₁R formation.

In the striatum, A_{2A}R and A₁R form heteromers and, under physiological conditions, adenosine preferentially activate A₁R,^{212,213} which controls glutamatergic neurotransmission, namely by a decrease in NMDAR-mediated responses.^{214,215} If a stoichiometric upsurge favoring nonheteromer forming A_{2A}Rs results in Gs-dominant signaling,¹⁷⁷ then an A_{2A}R assembly switch could be the primary event driving this pathological glutamate output. However, due to the physiological residual levels of A_{2A}R in the hippocampus and lack of experimental tools and techniques, the question of A₁R-A_{2A}R heterodimerization in glutamatergic synapses or a putative age-associated dimerization switch has not been yet solved.

Alzheimer's disease

An increase in hippocampal A_{2A}R expression is also observed in several other pathologies in humans, in which there is a clear correlation of hippocampal A_{2A}R upregulation with cognitive deficits, such as in Alzheimer's^{100,217} and Pick's disease.²¹⁸ Interestingly, adenosine levels are significantly increased in parietal and temporal cortices from the early stages of AD postmortem brains, alterations that occur independently of neurofibrillary tangles and β -amyloid plaques,¹⁶⁷ which would further enhance A_{2A}R activation and signaling.

Neuronal and astrocytic primary cultures incubated with A β -oligomers point out the therapeutic effect of A_{2A}R blockade. Either caffeine (nonselective adenosine receptor antagonist) or ZM241385 (selective A_{2A}R antagonist) prevented neuronal loss caused by incubation of A β_{25-35} in rat cultured cerebellar granule neurons.²¹⁹ In cortical primary astrocytes, treatment with SCH58261 rescues the decreased expression of glutamate transporters GLAST and GLT-1 and increased GFAP immunoreactivity caused by incubation with A β_{1-42} , suggesting that astrocytic A_{2A}R can therapeutically modulate the AD-associated increased levels of extracellular glutamate

and astrocytic reactivity.²²⁰ Importantly, these effects seem to be dependent of A_{2A}R since A β_{1-42} can no longer induce such astrocytic alterations in global-A_{2A}R-KO²²⁰ and, in this case, we cannot rule out neuronal A_{2A}R contribution in this A β -induced astrocytic dysfunction.

There is also compelling evidence from animal models of an aberrant A_{2A}R expression and signaling in AD. Intercerebroventricular injection of A β_{1-42} in mice and rats induces loss of nerve terminal markers and memory impairments, which were rescued upon either pharmacological blockade or genetic inactivation.²²¹ Neuronal A_{2A}R play an important role in this synaptic dysfunction, since SCH58261 also has a beneficial effect in preventing loss of synaptic markers in cultured hippocampal neurons incubated with the same A β fragment.²²¹ Opposite to what is described,²¹¹ A_{2A}R-mediated effects are dependent on p38 mitogen-activated protein kinase pathway and independent of cAMP/PKA,²²¹ suggesting that A_{2A}R may activate parallel and independent pathways that ultimately converge to induce synaptotoxicity.

The A_{2A}R-mediated effects on synaptic dysfunction are particularly visible in a mouse model of tauopathy, Thy-Tau22. A_{2A}R deletion is sufficient to prevent memory defects, LTD impairments, and Tau hyperphosphorylation observed in these animals.¹⁹⁵ Importantly, chronic treatment with the A_{2A}R antagonist MSX-3 rescued hippocampal-dependent memory even after onset of the pathology,¹⁹⁵ further emphasizing the central role of A_{2A}R in synaptic and memory dysfunction. Whether this is an astrocytic or neuronal A_{2A}R role remains inconclusive. The fact that Thy-Tau22-A_{2A}R^{-/-} mice exhibit decreased astrogliosis further supports the hypothesis of an astrocytic contribution to the A_{2A}R-mediated deficits, but does not discard a putative neuronal role. Consistent with this view, astrocytic A_{2A}R overexpression in AD human samples was assessed by correlation between A_{2A}R-encoding gene (*ADORA2A*) and *GFAP* messenger RNA (mRNA) levels and immunohistochemistry,²¹⁷ although neuronal expression was not addressed. Chemogenetic activation of astrocytic Gs-coupled signaling increases cAMP and CREB and reduces long-term memory in mice²¹⁷ and conditional ablation of A_{2A}R in astrocytes reduces memory deficits in 15–18 m.o. hAPP animals.²¹⁷

In APP/PS1 animals (double transgenic mice expressing a chimeric mouse/human amyloid precursor protein [Mo/HuAPP695swe] and a mutant human presenilin 1 [PS1-dE9]), an AD mouse model, lack of associative NMDAR-independent LTP in an early stage and cognitive impairments in the Y-maze test²²² are rescued upon pharmacological and viral A_{2A}R blockade in neurons.²²² Interestingly, either A_{2A}R or mGluR5 blockade rescues LTP back to wildtype (WT) levels, suggesting that A_{2A}R and mGluR5 operate through a common pathway to impair LTP, as we also observed.^{100,193} The crucial neuronal A_{2A}R role on synaptic dysfunction was further highlighted by us and others, since neuronal

A_{2A}R overexpression is sufficient to trigger CREB-dependent synaptic and memory dysfunction.^{100,223} We found that aged animals could be divided into two subsets: age-impaired animals, which performed worse than young rats in the Y-maze test, revealing no preference for the novel arm, and age-unimpaired animals performed within the range of young rats. Age-impaired animals seem to be distinguished by an LTD-to-LTP shift, whereas age-unimpaired animals could be distinguished by their lack of response to LFS. Consistent with an enhanced role of A_{2A}R upon aging, SCH 58261 decreased basal transmission in hippocampal slices of aged animals, while no effect was observed in young animals. A tendency toward an increased effect of SCH 58261 in age-impaired subset, when compared with age-unimpaired animals, suggests an increased A_{2A}R activation in age-impaired animals.¹⁰⁰

More specifically, a 3-week treatment with the selective A_{2A}R antagonist KW6002 restored memory impairments.¹⁰⁰ The fact that an acute A_{2A}R blockade is sufficient to rescue the LTD-to-LTP shift favors the hypothesis that A_{2A}R blockade reestablishes the physiological signaling of adenosine, rather than the receptor expression, which is unlikely to occur at such a short time frame. Accordingly, we have prior data showing that chronic KW6002 treatment rescues cognitive and synaptic impairments induced by stress, without altering A_{2A}R levels.²²⁴

Also, A_{2A}R activation increases Ca²⁺ influx via mGluR5 and NMDAR in primary neuronal cultures, discarding any major A_{2A}R astrocytic contribution.¹⁰⁰ Furthermore, we observed neuron-specific A_{2A}R staining and aged humans and AD patients' hippocampus,¹⁰⁰ consistent with previous data, in which single-cell polymerase chain reaction of laser-dissected cells of young rats revealed no A_{2A}R transcripts in GFAP positive cells.¹⁵³ Plus, in some brain pathological conditions, characterized by astrogliosis or increases in excitability, such as mesial temporal lobe epilepsy, there is a profound increase in A_{2A}R in astrocytes that is not related with A β or any other features of neurodegenerative disease.²²⁵ Interestingly, a recent study showed that deletion of A_{2A}R selectively in forebrain neurons prevented convulsions-induced neurodegeneration (decreased synaptic plasticity, loss of synaptic markers, and neuronal loss) in a kainate model of temporal lobe epilepsy.²²⁶ Altogether, these data strongly suggest that it is a synergism of astrocytic and neuronal A_{2A}R-mediated effects that defines the robust ability to control synaptic and memory dysfunction: synaptic dysfunction in aging and early AD may be driven predominantly by a neuronal A_{2A}R progressive dysfunction, whereas at later Braak stages of AD, astrocytic AD and inflammation may become more relevant.

Most importantly, this A_{2A}R pathological role was also observed in humans: AD patients exhibit an increase in A_{2A}R expression^{100,227} and a single nucleotide poly-

morphism (SNP) in *ADORA2A* was recently associated with episodic memory performance, hippocampal volume, and total tau in cerebrospinal fluid (CSF) in mild cognitive impairment (MCI) and AD patients,²²⁸ suggesting that this variation may affect A_{2A}R production. However, this still needs to be confirmed in future studies. Also, multiple prospective and retrospective studies emphasize the role of caffeine, a nonselective adenosine receptor antagonist, in slowing down cognitive decline in aged population and reducing the risk of developing AD.

Regulation of ADORA2A gene expression

An important question yet to be clarified is the mechanism by which A_{2A}R expression increases upon aging and AD on the hippocampus. Given the role of A_{2A}R in multiple brain regions, the regulation of its expression and signaling has received great attention. A 4.8 kb promoter-proximal DNA fragment in the *ADORA2A* confers selective expression in the CNS, but does not explain the strong expression observed in the striatum.²²⁹ These results suggest that the A_{2A}R expression is also controlled by other (epi)genetic factors that regulate the expression in each brain structure.

In fact, the coding for the A_{2A}R exists in two exons interrupted by one intron and has multiple promoters that lead to the production of various A_{2A}R transcripts.^{230,231} Each A_{2A}R transcript contains the same coding region plus an identical 3'untranslated region (UTR) and a distinct 5'UTR. This particular feature is conserved among species and might lead to the production of various transcripts with different 5'UTRs that control the transport, the translation efficiency, and the selection of the translational start site of the targeted transcript.^{232,233} Accordingly, lipopolysaccharide (LPS) stimulation of human polymorphonuclear neutrophils (PMNs) shifts A_{2A}R transcription from a longer 5'UTR to a shorter one, possibly increasing translation efficiency.²³¹ Therefore, different strategies in the transcriptional control of A_{2A}R may underlie differences in expression and signaling in physiological versus pathological conditions.

ADORA2A is a dual coding gene: in addition to the A_{2A}R protein, a 134-amino acid protein (uORF5) can be translated from an upstream open reading frame of the rat A_{2A}R gene.^{234–236} Expression of uORF5 was detected in rat striatum, associated with high A_{2A}R mRNA levels.²³⁷ In a rat pheochromocytoma line (PC12), uORF5 suppresses the activity of the transcription activator protein 1 (AP1) and regulates expression of proteins implicated in MAPK pathway.²³⁷ Interestingly, A_{2A}R activation led to a PKA-dependent increase in uORF5 protein levels at the posttranscriptional level, suggesting that uORF5 might act downstream of A_{2A}R and regulate A_{2A}R activity.²³⁷

Epigenetic A_{2A}R regulation. Several agents have been implicated in the epigenetic regulation of *ADORA2A*, including transcription factors (CREB, NF-1, NF κ B,

PPARgamma, YY-1, and ZBP-89), proinflammatory cytokines (IL-1 β and TNF-alpha), microRNA (miRNAs; miRNA-214, miRNA-34b, miRNA-15, and miRNA-16), and DNA methylation.^{238–251}

In polymorphonuclear leukocytes, the increase in A_{2A}R mRNA expression upon LPS stimulation correlates inversely with the expression levels of miRNA-214, miRNA-15, and miRNA-16.²⁴⁷ Reduced miRNA-34b levels were related to increased A_{2A}R levels in the putamen of PD patients, although alterations in miRNA-214, miRNA-15, and miRNA-16 were not found.²⁴³

Both genetic and pharmacological approaches revealed that DNA methylation by adenosine is receptor-independent. Genetic removal of ADK in the mouse forebrain, which leads to the elevation of intracellular adenosine, causes the reduction of DNA methylation. Chronic administration of an ADK inhibitor (5-iodotubercidin) in mice decreased global DNA methylation in the hippocampus of WT or A₁R KO mice.²⁵² Besides, liver-rescued adenosine deaminase (ADA) KO mice show elevated adenosine level and DNA hypomethylation in the placenta. A similar phenomenon is observed in liver-rescued ADA and *ADORA2B* double KO mice.²⁵³ Furthermore, an *in vitro* study showed that A_{2A}R (ZM 241385) and A_{2B}R (MRS1754) antagonism does not change adenosine-induced hypomethylation in human umbilical vein endothelial cells.²⁵⁴

Interestingly, although epigenetic effect of adenosine is receptor-independent, adenosine receptor gene(s) could also be epigenetically modified through global methylation. A previous study has identified three CpG islands in the 5'UTR region of human *ADORA2A*, which regulates its gene and protein expression. Buira *et al.* used different types of cell lines, including HeLa (epithelial cells), SH-SY5Y (neuroblastoma cells), and U87-MG (glioblastoma cells), which have distinct A_{2A}R mRNA expression levels to demonstrate the impact of DNA methylation on A_{2A}R expression. The endogenous A_{2A}R mRNA expression levels are inversely correlated with DNA methylation level in these cells.²⁴¹ DNA-methylation inhibitor (5-azacytidine) or activator (S-adenosyl-L-methionine) treatments increase or decrease A_{2A}R expression, respectively.²⁴¹ Furthermore, the same group observed the same phenomenon in the two cerebral regions (putamen and cerebellum) of the human brain.²⁴²

Besides DNA methylation, histone acetylation is a mechanism that allows tight but transient regulation of gene expression. Histone H3 acetylation is one of the most frequent epigenetic mechanism that increases the expression of the target genes by chromatin opening. We observed that chronic A_{2A}R blockade with KW6002 increased *ADORA2A* acetylation (unpublished data), consistent with the increase in A_{2A}R expression observed upon treatment,^{100,224} providing new insights into KW6002 mechanism of action.

In conclusion, these aspects of gene regulation upon aging are still poorly understood and surely deserve much more attention if one wants to pinpoint the age-related A_{2A}R expression shift which impacts on cognition. Very relevant steps were taken recently, when for the first time, a SNP in the *ADORA2A* gene was associated with episodic memory performance, hippocampal volume, and total tau in CSF in MCI and AD patients.²²⁸ This polymorphism occurs in a noncoding region, upstream to the coding sequence and it was just suggested, but not studied, that it could imply alterations in A_{2A}R expression.

Caffeine effects in aging and AD

Caffeine is the world's most popular psychoactive drug and is consumed by millions of people. While short-term CNS stimulating effects of caffeine are well-known,²⁵⁵ the long-term impact remains not completely clear. The Finland, Italy, and the Netherlands Elderly (FINE) Study showed that coffee intake was inversely associated with cognitive decline. In fact, elderly men (70 $\pm$ 10 years) who consumed coffee had a two times smaller 10-year cognitive decline than non-consumers.²⁵⁶ Accordingly, there was also an inverse association between the number of cups of coffee consumed per day and 10-year cognitive decline, with the least decline for men consuming three cups per day.²⁵⁶ Furthermore, in the Three City Study, with a sample of subjects aged 65 years and older, consumption of at least three cups of coffee per day was associated with less decline in verbal memory in women.²⁵⁷ On the opposite, coffee had no significant protective effect in women with less than two daily units.²⁵⁷

Importantly, other studies support a role for caffeine in the prevention of AD. A retrospective study reported an inverse correlation between coffee consumption and disease onset—AD patients had an average daily caffeine intake of 73.9 $\pm$ 97.9 mg during the 20 years before AD diagnosis, whereas the control had an average daily caffeine intake of 198.7 $\pm$ 135.7 mg during the corresponding 20 years of their lifetimes.²⁵⁸ In a prospective study, daily coffee drinking decreased the risk of AD by 31% during a 5-year follow-up.²⁵⁹ In line with those findings, moderate coffee drinkers (three to five cups of coffee per day) had a 65–70% decreased risk of dementia and a 62–64% decreased risk of AD compared with low coffee consumers.²⁶⁰ Furthermore, another prospective study showed that plasma caffeine levels at study onset were substantially lower (–51%) in MCI subjects who later progressed to dementia compared to levels in stable MCI subjects. Also, plasma caffeine levels >1200 ng/mL ($\approx$ 6 μ M) in MCI subjects were associated with no conversion to dementia during the ensuing 2/4-year follow-up period.²⁶¹ However, coffee and caffeine intake in midlife were not associated with cognitive impairment,

dementia, or individual neuropathologic lesions.²⁶² It is noteworthy that higher caffeine intake was associated with lower odds of having any neuropathological lesions at autopsy, including AD-related lesions, microvascular ischemic lesions, cortical Lewy bodies, hippocampal sclerosis, or generalized atrophy.²⁶²

The beneficial effects of caffeine in humans are not confined to AD. Epidemiological studies show an inverse relationship between the consumption of caffeine and the risk of developing PD^{263,264} and two *ADORA2A* polymorphisms (r71651683 and rs5996696) were inversely associated with PD risk.²⁶⁵ Although the mechanism was not directly addressed, the beneficial effects seem to be achieved via A_{2A}R blockade.^{266,267} Caffeine is metabolized primarily by cytochrome P450 1A2 (CYP1A2). An A to C substitution at position 163 in the CYP1A2 decreases enzyme inducibility. However, there is no association between this genetic alteration and caffeine consumption. In the brain, A_{2A}R activation has an important role in the stimulating and reinforcing properties of caffeine^{190,268} and A_{2A}R KO mice have less appetite for caffeine than WT littermates.²⁶⁹ A C to T substitution at position 1083 in the *ADORA2A* gene was associated with caffeine-induced anxiety among nonhabitual caffeine consumers²⁷⁰ and the probability of having such mutation decreases as the caffeine intake increases in a population, and people with that genotype are more likely to limit their caffeine intake.²⁷¹

Caffeine consumption has been shown to improve the memory performance in multiple AD mice models, ascertaining its protective properties against cognitive impairment and in favor of improved memory retention (see Kolahdouzan and Hamadeh²⁷²). In aged rodents, perfusion of caffeine rescued neuronal A_{2A}R-driven synaptic plasticity shift in the hippocampus,¹⁰⁰ while having no effects in excitatory CA1 currents in young animals.¹⁹⁴ In aged animals, chronic intake of caffeine for 12 months prevents age-associated recognition memory decline, possibly by restoring BDNF signaling.²⁷³ Chronic caffeine consumption also rescued synaptic and memory impairments in a mouse model of chronic unpredictable stress in a manner similar to selective A_{2A}R antagonist (KW6002) administration or A_{2A}R genetic deletion selectively in neurons,²⁷⁴ being that stress is a physiopathological condition associated with upsurge of A_{2A}R and hippocampal dysfunction.^{224,274} Memory deficits were prevented by caffeine in transgenic mouse models of Alzheimer's disease, both amyloid and Tau-based.^{195,275–277}

The caffeine-mediated beneficial effects also included decrease in several proinflammatory (CD68, CD45, TLR2, CCL4, and TNF α) and oxidative stress (Nrf2, MnSOD, and EAAT3) markers found upregulated in the hippocampus of THY-Tau22 animals.²⁷⁸ Interestingly, caffeine reduces tau phosphorylation in different residues from the ones observed with A_{2A}R antagonist genetic deletion^{195,278} and decreases the amount of Tau proteolytic fragments,²⁷⁸ a pathological feature that is

not influenced by genetic deletion.¹⁹⁵ These results suggest that regulation of Tau by caffeine may result from a wider range of actions than from inhibition of A_{2A}R function alone.

Conclusion

In summary, age-related memory impairments are explained by changes in neuronal and synaptic morphology and function, namely reduction in the amplitude of the Schaffer collaterals-induced fEPSP and in PSD area in CA1.^{62–64} These results, associated with maintenance of the unitary EPSP size,⁶⁶ suggest that aging might not be associated with alterations in the strength of individual synaptic connections, but instead with an increase in nonfunctional or silent synapses in the hippocampus.

The changes observed at individual synapses directly affect synaptic plasticity mechanisms, namely an alteration in the susceptibility to induce LTP and LTD due to a shift in Ca²⁺-dependent induction mechanisms.^{84,99} Together with impairments in calcium buffering and influx mechanisms, such as via NMDAR and L-type VDCC (voltage-dependent calcium channels),^{103,111,112,121–125} these observations support the *calcium hypothesis* of aging, which implicates raised intracellular Ca²⁺ as the major source of functional impairment and degeneration in aged neurons.^{104–106}

Caffeine is the world's most popular psychoactive drug and is consumed by millions of people. Multiple prospective and retrospective studies emphasize the role of caffeine, adenosine receptor antagonist, in slowing down cognitive decline in aged population and reducing the risk of developing AD. Although the mechanism is not yet disclosed, the beneficial effects seem to be achieved via A_{2A}R blockade.^{266,267}

A_{2A}R expression and signaling is profoundly altered in the hippocampus upon aging. A_{2A}R density and coupling to G protein is increased,^{100,144,197–199} probably enhancing the efficiency of this receptor to facilitate neurotransmitters release in glutamatergic synapses by a presynaptic mechanism.¹⁹⁷ Interestingly, neuronal A_{2A}R overexpression in the same magnitude to the one observed in human aging is sufficient to trigger synaptic and cognitive deficits, due to a mGluR5-dependent NMDAR overactivation and linked to enhanced Ca²⁺ influx,¹⁰⁰ which recapitulates the main synaptic alterations observed upon aging. Importantly, either A_{2A}R pharmacological blockade or genetic deletion prevents synaptic and memory impairments in aged rodents and several AD models.^{100,222,223,278}

Because of the diversity and complexity of the adenosine receptor-dependent and independent regulatory mechanisms, a better understanding of the precise mechanism of adenosine and its receptors in pathophysiological conditions in different brain regions and CNS cell types will allow the development of novel therapeutic strategies for synaptic and memory dysfunction upon aging and AD.

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Authors' Contributions

M.T.-F. has written the article. M.T.-F., J.E.C., P.A.P., and L.V.L. discussed the article. All authors have reviewed and approved the article.

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