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Early synaptic deficits in the APP/PS1 mouse model of Alzheimer’s disease involve neuronal adenosine A2A receptors

Silvia Viana da Silva1,2, Matthias Georg Haberl3,*, Pei Zhang1,*, Philipp Bethge1, Cristina Lemos4, Nélio Gonçalves4, Adam Gorlewicz1, Meryl Malezieux1, Francisco Q. Gonçalves4, Noëlle Grosjean1, Christophe Blanchet1, Andreas Frick3, U Valentin Nägerl1, Rodrigo A. Cunha4,5 & Christophe Mulle1

Synaptic plasticity in the autoassociative network of recurrent connections among hippocampal CA3 pyramidal cells is thought to enable the storage of episodic memory. Impaired episodic memory is an early manifestation of cognitive deficits in Alzheimer’s disease (AD). In the APP/PS1 mouse model of AD amyloidosis, we show that associative long-term synaptic potentiation (LTP) is abolished in CA3 pyramidal cells at an early stage. This is caused by activation of upregulated neuronal adenosine A2A receptors (A2AR) rather than by dysregulation of NMDAR signalling or altered dendritic spine morphology. Neutralization of A2AR by acute pharmacological inhibition, or downregulation driven by shRNA interference in a single postsynaptic neuron restore associative CA3 LTP. Accordingly, treatment with A2AR antagonists reverts one-trial memory deficits. These results provide mechanistic support to encourage testing the therapeutic efficacy of A2AR antagonists in early AD patients.
Loss of episodic hippocampal-dependent memory is the earliest clinical sign of Alzheimer’s disease (AD), consistent with reduced activation of hippocampal regions during memory encoding tasks in patients with mild cognitive impairment. Synaptic loss is the best morphological correlate of cognitive impairment in early AD, rather than amyloid-beta plaques, tangle formation or neuronal loss. The CA3 subregion of the hippocampus encodes episodic memories, particularly at the earliest stage of acquisition, presumably by developing instant representations of a context. The autoassociative network of recurrent connections among CA3 pyramidal cells (PCs) is thought to enable the storage of episodic memories through synaptic plasticity of these associative/commissural (A/C) inputs. In mouse models of AD, synaptic dysfunction has been mostly studied in the CA1 region or dentate gyrus with very few studies addressing deficits in CA3 (ref. 6). Long-term potentiation (LTP) of synaptic transmission at Schaffer collateral-CA1 synapses is generally impaired in mouse models of AD (refs 6,7). Whether synaptic plasticity is affected in the recurrent CA3 network in AD mouse models has not yet been addressed.

Dysregulation of NMDA receptors (NMDAR) has been proposed as a link between Aβ accumulation and disruption of LTP (ref. 8) although neuromodulation systems may also be impaired. Epidemiologic studies indicate that regular caffeine intake attenuates memory decline during aging and reduces the risk to develop AD (ref. 10). In animal models of AD, chronic caffeine intake prevents memory deterioration, an effect mimicked by the selective inhibition of A2A receptors (A2AR), which are a main target of caffeine. Conversely, the overactivation of hippocampal A2AR is sufficient to disrupt memory performance. A2AR are upregulated in cortical areas of AD patients and A2AR show increased expression in the hippocampal formation, but the mechanisms by which the blockade of A2AR restores memory impairment are not understood. The impact of A2AR may depend on the stage of progression of the disease, with a role for astrocytic A2AR at late stages. Here we provide evidence for early synaptic dysfunction in CA3 PCs in a mouse model of AD, and we explore the implication of NMDAR and A2AR.

**Results**

**Impaired A/C synaptic plasticity in CA3 in APP/PS1 mice.** We performed whole-cell patch clamp recordings of CA3 PCs to characterize the synaptic properties of A/C inputs in 6-month-old male APP/PS1 (Amyloid precursor protein (APP) gene with Swedish mutation and presenilin 1 gene (PS1)) with deletion of exon 9) mice at early stages of amyloid-beta deposition when the CA3 region appears largely spared (Supplementary Fig. 1a). We initially recorded excitatory postsynaptic current (mEPSCs), which arise from the different types of glutamatergic synapses impinging on CA3 PCs. The average amplitude of mEPSCs was significantly decreased in APP/PS1 compared with wild-type (wt) mice (wt: 29 ± 2 pA, APP/PS1: 23 ± 1 pA, P = 0.007), whereas mEPSC frequency, measured by the inter event interval (IEI), was only minimally affected (wt: 0.7 ± 0.2 s, APP/PS1: 0.9 ± 0.1 s, Supplementary Fig. 2a,b). The decreased amplitude was mainly attributed to mEPSCs with amplitudes <50 pA, suggesting that large amplitude mEPSCs arising from mossy fibre-CA3 synapses were not affected (Supplementary Fig. 2b). We further found that the paired-pulse ratio (PPR) of A/C synaptic responses was not different between APP/PS1 (1.9 ± 0.1) and wt mice (1.8 ± 0.1), arguing against presynaptic alterations (Fig. 1a–c). Pairing of presynaptic activation and postsynaptic depolarization induces an NMDAR-dependent LTP of A/C inputs in CA3 PCs (ref. 17). Pairing A/C stimulation (100 stimuli at 2 Hz) with a depolarization to 0 mV (Fig. 1a) induced a robust LTP of AMPA-EPSCs in wt mice (218 ± 38%), which was absent in APP/PS1 mice (93 ± 12%, P < 0.0001; Fig. 1d–f).

To explore the mechanisms underlying the abolition of LTP of A/C inputs in APP/PS1 mice, we first tested whether this loss was correlated with morphological alterations of CA3 dendritic spines. Bilateral stereotaxic injections of retrograde rabies virus expressing green fluorescent protein (GFP; RAVB) into CA1 were performed to specifically label CA3 PCs. We quantified spine density and morphology in these neurons using stimulated emission depletion (STED) microscopy. We found a decrease in the density of spines in the stratum radiatum of APP/PS1 mice (wt: 11.5 ± 1.1 spines per 10 μm, APP/PS1: 8.7 ± 0.6 spines per 10 μm, P = 0.037; Fig. 2a,b). STED microscopy allowed us to examine traditionally neglected key nanoscale features of spine morphology (Supplementary Table 2). Although spine length was similar in both genotypes (wt: 0.82 ± 0.02 μm, APP/PS1: 0.81 ± 0.02 μm, Fig. 2c), we found a marked shift to larger spine heads in APP/PS1 mice (wt: 0.44 ± 0.01 μm, APP/PS1: 0.49 ± 0.01 μm, P < 0.0001, Fig. 3d), in parallel with shorter and wider spine necks (Fig. 2e,f and Supplementary Table 2). As synapse compartmentalization is strongly shaped by spine morphology, these changes (Fig. 2g) could potentially explain the decreased ability of the pairing protocol to induce LTP. However, the compartmentalization factor, which is a measure of biochemical compartmentalization of spine synapses (see definition in the Methods section), was preserved because the effects of the structural changes cancelled each other out (Fig. 2h). Thus the morphological phenotype seems insufficient to explain the absence of LTP observed at A/C synapses in APP/PS1 mice.

**Loss of A/C LTP is not associated with alterations of NMDAR.** We tested whether dysregulation of NMDAR function, which has been implicated in impaired synaptic plasticity in models of AD (ref. 8), may be causally related to the loss of LTP in APP/PS1 mice. Insufficient membrane potential depolarization can be ruled out as a possible cause for the loss of A/C LTP in APP/PS1 mice as our LTP protocol controls for postsynaptic membrane potential. We found no difference in synaptic NMDAR/AMPAR ratio between wt (36 ± 4%) and APP/PS1 mice (37 ± 5%; Fig. 3a,b). However, the relative expression of different GluN2 NMDAR subunits at synapses may strongly modulate plasticity, and the toxic effects of amyloid-beta oligomers applied in cultured neurons and acute slices is thought to involve the GluN2B subunit. We found no difference in the inhibition of NMDAR EPSC amplitude by Ro25-6981 (1 μM), a selective antagonist of GluN2B-containing NMDAR (wt: 59.0 ± 5.9%, APP/PS1: 73.6 ± 7.6%; Fig. 3c,d) or in NMDAR EPSC decay time (Supplementary Fig. 3a,b), ruling out a major change in GluN2B subunit composition of synaptic NMDAR in CA3 PCs in APP/PS1 mice. The subcellular localization of NMDAR (synaptic versus extrasynaptic) leads to the activation of different intracellular signalling pathways; importantly, extrasynaptic NMDAR were shown to be essential for amyloid-beta mediated toxicity. We evaluated extrasynaptic NMDAR by measuring the amplitude of tonic NMDAR-mediated currents recorded at +40 mV. Tonic currents were blocked to the same extent by the NMDAR antagonist D-AP5 (50 μM) in both genotypes (wt: 34 ± 4 pA and APP/PS1: 30 ± 5 pA; Fig. 3e,f). In addition, the blockade of tonic currents by Ro25-6981 (1 μM) did not indicate any change in GluN2B content (Supplementary Fig. 3c,d). Finally, we reasoned that if dysregulation of NMDAR was responsible for...
Figure 1 | Early synaptic alterations in 6-month-old APP/PS1 CA3 PCs. (a) Scheme illustrating the hippocampus with a recording electrode on a CA3 PC (R) and a stimulating electrode (S) in the stratum radiatum. Depo-pairing protocol used to trigger NMDAR-dependent LTP: depolarization of postsynaptic cell to 0 mV paired with 100 stimuli at 2 Hz frequency. (b) Example traces of paired-pulse responses (40 ms interval, average of 5 sweeps) in cells from both genotypes. (c) Bar graph summarizing PPR values in wt (n=21) and APP/PS1 mice (n=25; P=0.054, unpaired t-test). (d) Sample traces representing average A/C-EPSCs 10 min before and 30 min after depo-pairing protocol. (e) Summary time course of normalized A/C-EPSCs in the experiments illustrated in d. (f) The robust LTP of A/C synapses found in 6-month-old wt mice (n=12) is abolished in APP/PS1 mice (n=11, ***P<0.0001, unpaired t-test; Supplementary Table 1). Recordings were performed in the presence of 10 μM bicuculline and 3 μM CGP55845.

Figure 2 | Alterations in A/C spine morphology. (a) Representative STED images obtained from RABVΔG-GFP(RG) infected neurons. Scale bar, left panel, 10 μm; right panel, 1 μm; inset, 250 nm. (b) Bar graph summarizing the reduction of spine density. Several branches of distal dendrites of CA3 PCs were analysed in wt (n=8 branches, 4 mice) and in APP/PS1 mice (n=9 branches, 4 mice; *P=0.037, unpaired t-test). (c) Cumulative distribution illustrating the absence of any difference in spine lengths between both genotypes (Kolmogorov-Smirnov (KS) test P=0.144). Number of spines analysed was equal for graphs from c to h, n=300 for wt and n=288 for APP/PS1, from four mice for each genotype. (d) Cumulative distribution of spine head width (KS-test ***P<0.0001), (e) spine neck length (KS-test *P=0.015) and (f) spine neck width (KS-test ***P<0.0001) from wt and APP/PS1 mice. (g) Schematic spines illustration of morphological alterations observed in A/C spines with mean values indicated. (h) The cumulative distribution of the compartmentalization factor calculated for each spine was not different between wt (1.35±0.13) and APP/PS1 mice (1.35±0.10, P=0.063, KS-test).
the loss of A/C LTP in APP/PS1 mice, then the selective potentiation of synaptic NMDAR by d-serine may rescue plasticity. Enhancement of synaptic NMDAR by bath application of 10 μM d-serine (~20% increase in current amplitude, Supplementary Fig. 3e–g) did not rescue A/C LTP in APP/PS1 mice (109.0 ± 9.5%, Supplementary Fig. 3h–j and Supplementary Table 1) or alter A/C LTP in wt mice. Hence, the complete loss of A/C LTP in 6-month-old APP/PS1 mice does not appear to correlate with alterations in the function or GluN2B content of neither synaptic nor extrasynaptic NMDAR.

Inhibition of A2AR rescues A/C LTP in APP/PS1 mice. A2aR control synaptic plasticity, are involved in memory impairment and are upregulated in the brain of AD patients and animal models of AD. We used a binding assay on isolated CA3 synaptic membranes and showed a robust increase of A2aR density in 6-month-old APP/PS1 mice (wt: 38 ± 7 fmol per mg protein; APP/PS1: 75 ± 6 fmol per mg protein, P = 0.002; Fig. 4a). Based on these results, we investigated if a short incubation of the slices for 10 min with the selective A2aR antagonist SCH58261 (50 nM) could affect synaptic plasticity at A/C synapses. Strikingly, SCH58261 rescued in large part A/C LTP in APP/PS1 synapses (160 ± 16%), in comparison with APP/PS1 slices treated with vehicle solution (98 ± 9%, Fig. 4b–d).

ZM241385 (50 nM), a chemically distinct and selective A2aR antagonist was equally effective in rescuing A/C LTP (wt: 165.9 ± 20.8%, P = 0.063; Fig. 4d and Supplementary Table 1). The difference in mEPSCs amplitude observed between APP/PS1 and wt littermates (wt: 25.2 ± 1.5 pA; APP/PS1: 19.5 ± 1.1 pA) was not rescued by a short incubation with SCH58261 (wt: 24.0 ± 1.5 pA; APP/PS1: 19.6 ± 0.8 pA; P = 0.006 for genotype effect; Supplementary Table 2). Similarly, the distribution of mEPSCs amplitudes in both genotypes was not altered by SCH58261 (Supplementary Fig. 4b). Although SCH58261 incubation caused a small alteration in the distribution of IEIs between mEPSCs (Supplementary Fig. 4d), it did not cause any significant effect on the mean IEI values (wt: 0.15 ± 0.1 s; APP/PS1: 0.19 ± 0.1 s; Supplementary Table 1). Pharmacological inhibition of either A2aR or mGluR5 did not fully rescue A/C LTP, possibly because the antagonists attenuate LTP in wt mice (Supplementary Table 2).

A2aR act synergistically with mGluR5 in hippocampal neurons, and mGluR5 antagonists rescue contextual fear conditioning in APP/PS1 mice. Accordingly, we found that a selective antagonist of mGluR5 (MTEP, 10 μM) also rescued A/C LTP levels in APP/PS1 mice to values similar to those obtained with the A2aR antagonist SCH58261 (164 ± 28%, Fig. 5a–c). A combined incubation with both MTEP and SCH58261 did not further increase A/C LTP levels in 6-month-old APP/PS1 mice (163 ± 18%, P = 0.008, Fig. 5a–c and Supplementary Table 1), suggesting that A2aR and mGluR5 operate through a common pathway to impair LTP. Importantly, these antagonists did not further increase the level of A/C LTP in wt mice (Supplementary Fig. 5a–c and Supplementary Table 1). Pharmacological inhibition of either A2aR or mGluR5 did not fully rescue A/C LTP, possibly because the antagonists attenuate LTP in wt mice (Supplementary Table 2).

Adenosine neuromodulation depends on a balanced activation of inhibitory A1R and A2AR. We tested whether adenosine A1R levels were comparatively affected by using a binding assay on purified CA3 synaptic membranes and observed a modest increase of A1R density in 6-month-old APP/PS1 mice (wt: 938.2 ± 27.2 fmol per mg protein; APP/PS1: 1044.0 ± 19.0 fmol per mg protein, P = 0.002; Supplementary Fig. 6a). To test if this alteration in A1R density affected A/C synapses, we recorded evoked AMPAR EPSCs and bath applied 100 nM DCCPX (a selective A1R antagonist). DCCPX equally increased the amplitude of AMPAR EPSCs in APP/PS1 (134.0 ± 11.0%) and wt mice (143.3 ± 17.8%, Supplementary Fig. 6b–d), arguing for similar levels of A1R at those synapses.
APP/PS1 vehicle
APP/PS1 SCH58261
APP/PS1 ZM241385

Figure 4 | Blockade of neuronal A2AR restores synaptic plasticity in CA3 PCs. (a) The density of A2AR is increased in synaptic membranes prepared from the CA3 region of APP/PS1 mice compared with wt littermates (n = 6, **P = 0.002, Mann–Whitney test). (b) Example traces representing a 10 min average of A/C-EPSCs before and 30 min after depo-pairing LTP protocol in the presence of SCH58261 (50 nM) or ZM241385 (50 nM). (c) Summary time course of normalized A/C-EPSCs recorded from APP/PS1 mice during LTP protocol performed in the presence of the two different A2AR antagonists or in control conditions. (d) Bar graph representing mean LTP amplitude recorded at 30–40 min after depo-pairing protocol represented in (c). A 10-min incubation period with two different classes of A2AR antagonists, SCH58261 (n = 11) and ZM241385 (n = 12, P = 0.006 Kruskal–Wallis test with Dunn’s multiple comparison test), rescued the LTP of A/C synapses in APP/PS1 mice (n = 11 for vehicle, Supplementary Table 1). Electrophysiology recordings were performed in the presence of 10 μM bicuculline and 3 μM CGP55845.

A2AR antagonists can prevent Aβ-induced memory impairment in mice.26 We thus tested SCH58261 (intraperitoneal injection, 0.1 mg per kg, for 6–7 days) could reverse deficits in one-trial memory tasks, which depend on CA3 circuits2,4, in a different group of 6-month-old APP/PS1 mice. When tested in the object displacement paradigm (30 min inter-trial interval), APP/PS1 mice showed impaired recognition of a displaced object, which was rescued on A2AR inhibition (Fig. 6a–c). Wt mice treated with saline displayed a higher displacement index (68.2 ± 4.0%), whereas APP/PS1 mice did not show any preference for the displaced object (54.9 ± 4.1%, P = 0.034 for genotype effect, Fig. 6b). This difference between genotypes was abrogated by SCH58261 treatment (wtSCH58261: 63.5 ± 3.3%; APP/PS1SCH58261: 60.0 ± 3.7%). Likewise, A2AR inhibition rescued the performance of APP/PS1 mice in a modified Y-maze task (30 min inter-trial interval), as shown by the increase in the percentage of time exploring the novel arm (wtSaline 40.5 ± 2.4 and APP/PS1Saline: 34.6 ± 2.8%, P = 0.024 for genotype effect, Fig. 6d–f). APP/PS1 mice treated with SCH58261 showed no difference in the time spent in the novel arm (42.7 ± 4.1%) when compared with wt mice treated with SCH58261 (46.9 ± 2.7%, Fig. 6e).

A2AR expressed in CA3 PCs are involved in A/C LTP deficits. Since both neuronal12 and astrocytic A2AR (ref. 14) may control memory performance, we sought to understand if the pharmacological rescue of plasticity at early stages of AD is either due to an action on neurons or glia. For this purpose, we employed a knockdown approach of A2AR in neurons using a VSV-G-coated lentivirus expressing shRNA against A2AR and a fluorescent marker (shA2AR, see validation in Supplementary Information). One month after stereotaxic infection in the CA3 region of 5-month-old APP/PS1 mice, a few GFP-positive CA3 PCs (shA2AR+), expressing the shRNA) could be identified in hippocampal slices (Fig. 7a). To check for neuronal tropism of the lentiviral construct, we injected eGFP-shA2AR lentivirus in the CA3 region of wt mice and performed immunohistochemistry on hippocampal sections. We observed a near exclusive co-localization with the neuronal marker NeuN for virally injected eGFP-positive cells, whereas no co-localization was observed with the astrocytic marker glial fibrillary acidic protein (GFAP) (Supplementary Fig. 7).

We found that genetic silencing of A2AR in individual CA3 PCs rescued LTP of A/C synapses in these cells in 6-month-old APP/PS1 mice.
PS1 mice. LTP of A/C synapses did not recover in uninfected neighbouring sh-A2AR CA3 PCs (sh-A2AR: 174 ± 20%, sh-A2AR cells: 92 ± 9%; P = 0.001; Fig. 7a–c) or in cells infected with scramble RNA (scrRNA: ± 105 ± 8%, Fig. 7d–f). A/C LTP could be rescued in scrRNA+ cells with a short SCH58261 incubation as in control APP/PS1 CA3 PCs (168 ± 6%, P = 0.0014, Fig. 7d–f). Furthermore, the extent of A/C LTP following SCH58261 incubation was not different between scrRNA+ and shRNA+ cells (173 ± 15%), indicating that the A2AR shRNA occludes the effect of SCH58261 in shRNA+ cells and not in control neurons (Supplementary Table 1 and Fig. 7i). This strongly suggests that the loss of A/C LTP is caused by gain of function of A2AR specifically in neurons and not glial cells at this early stage of AD. In addition, removal of A2AR from a single postsynaptic CA3 PC is sufficient to rescue A/C LTP, strongly suggesting a pathological function of postsynaptic A2AR.

Discussion
This study provides the first characterization of AD-related synaptic impairment in the autoassociative network of recurrent connections between CA3 PCs which is crucially involved in the initial encoding of memory3,5. At early stages of AD pathology in APP/PS1 mice, we found no major alteration of basal AMPAR or NMDAR-mediated transmission. However, we show that associative pairing between presynaptic A/C stimulation and postsynaptic spiking failed to induce LTP in single CA3 PCs. Studies of LTP in mouse models of AD have often provided contradictory results6, possibly depending on the age and on the experimental conditions to induce plasticity. In CA1, LTP of field EPSPs induced by high-frequency stimulation is attenuated—but not abolished—in an age-dependent manner in 4–6-month-old APP/PS1 mice31. Spine shape may determine the ability of synapses to undergo LTP by tuning biochemical and electrophysiological compartmentalization79. We observed a moderate decrease of spine density, which may correlate with subtle differences in the frequency of mEPSCs but cannot explain the abolition of LTP. Using STED microscopy, we unravelled subtle changes in spine necks and spine heads in 6-month-old APP/PS1 mice,34 which may contribute to the modulation of plasticity. Despite these changes, the morphological changes are not strong enough to explain the loss of LTP in APP/PS1 mice. As NMDAR are well known to be a target of Aβ oligomers, the decreased ability of A/C synapses to undergo LTP may be due to a decrease of NMDAR in the spine head. In line with our data, chronic blockade of GluN2B-containing NMDAR does not rescue behaviour or spine morphology in PS2APP mice34. We do not exclude the possibility that NMDAR
signalling may be strongly compromised at later stages of AD. However our study suggests that NMDAR may not be a valid target to restore hippocampal synaptic plasticity and the moderate cognitive deficits at early stage of AD pathology, in support of the lack of clinical efficiency of memantine in mild AD (ref. 35).

Strikingly, we found that the acute inhibition of A2AR by two different antagonists rescued LTP of A/C synapses, strongly suggesting that activation of A2AR by ambient adenosine or by adenosine produced during the LTP induction protocol disrupts synaptic plasticity in APP/PS1 mice. This raises several questions with regards to the source of adenosine, the cellular localization of A2AR and the signalling mechanism engaged to compromise LTP. On brain injury, A2AR undergo increased expression in both neurons and glia14,36; their conditional removal from astrocytes ameliorates spatial memory selectively at later stages of AD (ref. 14). Our pharmacological experiments do not allow distinguishing effects of the antagonists on glial cells, pyramidal neurons or interneurons. However, we found that A2AR are upregulated in CA3 synapses at early stages of AD. Moreover, silencing A2AR selectively in an individual CA3 PC is sufficient to restore A/C LTP. Thus, the increase of A2AR in postsynaptic CA3 PCs can by itself explain the abolition of LTP. Although we cannot rule out an increased expression of astrocytic A2AR at this stage, this is not required for impaired plasticity. Interestingly, the prevention of synaptic plasticity can be relieved by acute antagonism of A2AR, providing some potential therapeutic strategies for early cognitive dysfunction in AD. Accordingly, the treatment of APP/PS1 mice with SCH58261 improved their one-trial spatial memory performance in both an object displacement and a modified Y-maze task, which is thought to depend on the activity of CA3 circuits5. More work is necessary to establish clear links between impaired A/C LTP and rapid acquisition of memory. Systemic administration of SCH58261 did not affect recall in one-trial memory tasks in wt mice, although it partly inhibited A/C LTP. Conversely, in APP/PS1 mice, behavioural deficits were markedly improved by SCH58261 administration, although A/C LTP deficit was only partly reverted.

The mechanism by which activation of upregulated A2AR compromises LTP is unknown. Because silencing A2AR in an individual CA3 PC rescues LTP, postsynaptic A2AR are likely at play. A2AR are pleiotropic receptors activating multiple G proteins and transducing pathways; the extended C-terminal domain of A2AR engages several proteins other than those canonically involved in signalling by G-protein coupled receptors37. Furthermore, the signalling pathways of upregulated A2AR in disease-like conditions may be altered38. Which of these multiple transducing systems is associated with the control by A2AR of synaptic plasticity is still unknown.
The ability of A2A R and mGluR5 to disrupt A/C LTP is intriguing and may provide a clue to the underlying mechanism\textsuperscript{29}. Interestingly, A2A R and mGluR5 are both also necessary for the expression of LTP of NMDARs in CA3 PCs (ref. 39). The molecular mechanisms by which the amount of A2A R is increased at early stages in neurons and at later (neuroinflammatory) stages in astrocytes still remains to be deciphered, in view of the complexity of the A2A R gene promoter\textsuperscript{40}. Upregulation of A2A R at early stages may not be restricted to CA3 PCs and may include interneurons and interneuronal connections to regulate the overall excitability of CA3 circuits\textsuperscript{41}. Nevertheless, postsynaptic A2A R in CA3 PCs, rather than such possible alterations account for deficits in A/C CA3 LTP.

Overall, the present study shows that at the early onset of AD-like features in APP/PS1 mice, associative long-term synaptic plasticity is abolished in CA3 PCs due to the activation of upregulated A2A R in the postsynaptic compartment rather than to modifications of synaptic structure or NMDAR function. Our results are based on one mouse model of AD, and it would certainly be important in the near future to extend this to other experimental models of cognitive deficits. Nonetheless, the exquisite ability of A2A R blockade to restore the defective A/C LTP in APP/PS1 mice, in parallel to studies linking A2A R to cognitive deficits\textsuperscript{11,12}, provides an additional mechanistic support to encourage testing the therapeutic efficacy of A2A R antagonists in early AD patients.

**Methods**

**Mice.** APP/PS1 mice were obtained from Jackson’s Lab and used according to regulations of the University of Bordeaux/CNRS Animal Care and Use Committee. The colony was maintained in a hemizygote state by crossing transgenic female mice to B6C3F1/J male mice. Throughout all their life, littermate wt and APP/PS1 mice were anaesthetized with a ketamine (75 mg kg\textsuperscript{-1}) and xylazine (10 mg kg\textsuperscript{-1}) mix and intracardially perfused with ice-cold oxygenated cutting solution composed of 200 mM of sucrose, 20 mM glucose, 0.4 mM CaCl\textsubscript{2}, 8 mM MgCl\textsubscript{2}, 2 mM KCl, 1.3 mM NaH\textsubscript{2}PO\textsubscript{4}, 26 mM NaHCO\textsubscript{3}, 1.3 mM ascorbate, 0.4 mM pyruvate and 3 mM kynurenic acid (pH 7.3). When the solution coming from the brain was live, it was decapsitated and the head immersed in ice-cold cutting buffer. The brain was quickly dissected and parasagittal slices (350 μm thick) were cut on a vibratome. The slices were then transferred into another resting aCSF, now without kynurenic acid and left at room temperature for a maximum of 6 h after cutting. All drugs from Sigma-Aldrich, unless otherwise stated (see Supplementary Table 3 for details).

Cells were identified by differential interference contrast microscopy using an Olympus fixed stage upright microscope (BX51WI) equipped with a × 40 magnification immersion objective. Once at the electrophysiology set-up, slices were superfused with oxygenated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) aCSF composed by 125 mM NaCl, 2.5 mM KCl, 2.3 mM CaCl\textsubscript{2}, 1.3 mM MgCl\textsubscript{2}, 1.25 mM NaH\textsubscript{2}PO\textsubscript{4}, 26 mM NaHCO\textsubscript{3} and 14 mM glucose (pH 7.3) and with 10 μM bicuculline. A period of 10 min was allowed for slice stabilization and removal of the excess Mg\textsuperscript{2+} from the resting solution. Whole-cell recordings were made at room temperature from visually identified CA3 PCs with borosilicate glass capillaries with 3−5 MΩ resistance filled with 140 mM NaCl, 2 mM MgCl\textsubscript{2}, 4 mM NaCl, 0.2 mM EGTA, 5 mM phosphocreatine, 0.4 mM ATP and 10 mM HEPES (pH 7.2). The access resistance was measured by the ionic current injected at 0 mV and the resistance tested to change by >20%. EPSCs were evoked using a patch pipette (≈5 MΩ) filled with aCSF positioned in the CA3 stratum radiatum. The identification of the A/C synaptic currents was performed according to the following criteria: (1) frequency facilitation not >1.2 when switching stimulation from 0.1 to 1 Hz, (2) paired-pulse facilitation ratio <3 and (3) EPSCs decays free of a secondary peak that might indicate the presence of polysynaptic contamination. No liquid-junction potential correction was used. Recordings were made using an EPC10.0 amplifier (HEKA Elektronik), filtered at 0.5−1 kHz and analysed using IGOR Pro and Neuramatic V2.6 software.

**Stereoactive injections and viral vectors.** A small hairpin (sh) RNA was engineered to target the mouse A2A R (shA2AR-RNA), with the following sequence: 5′-CTA GTT TTC AAA AAG AAC AAC TGC AGT CAA AAAAA TTG CGT ACT GCA GTT-3′ and 5′-CGG GGA TCT GTG TCT GTA CAC AGA AC-3′. These oligomers and the H1 forward primer 5′-CAC CGA AGC AGC TGA TCA ACC CG-3′ were used for PCR with the pBcH1 plasmid (pBC; University of Bordeaux, Leiden, The Netherlands). The resulting vector was then packaged in Invitrogen’s Lentivector System. Lentivectors were produced in HEK293T cells, with a four-plasmid system, as previously described\textsuperscript{42} and the lentiviral particles content was determined by assessing HIV-1 p24 antigen levels (Gentaur, Spain). Viral stocks were stored at −80 °C until use and were thawed on ice before their in vivo administration. For the in vivo experiments, the lentivectors were administered to mice anaesthetized with avertin (240 μg g\textsuperscript{-1}), i.p. and placed in a stereotoxic frame (Stoelting, Wood, Dale, USA). To test the in vivo efficiency of the shA2AR-RNA, we first injected the lentivector (286,000 ng of p24 antigen per ml) unilaterally into the mouse striatum, where the high density of A2A R allows a faithful quantification of shA2AR-RNA expression. Mice received 1.4 μl of shA2AR-RNA or scRNA (0.2 μl per min) with an automatic injector (Stoelting) in the following coordinates: antero-posterior: ±0.6 mm; medio-lateral: ±1.8 mm; and ventral: ±3.3 mm. After 3 weeks, an immunohistochemical analysis of the percentage striatal area labelled with eGFP (ref. 43) showed that the lentivectors transfected 27.2 ± 2.7% (n = 4) of the striatal dorsal striatum (D1). A 3D reconstruction of each A2A R mRNA expression, evaluated by quantitative PCR (ref. 44) and a 55 ± 6% (n = 4) decrease of A2A R protein density in the striatum, as evaluated by Western blot analysis\textsuperscript{45}. Mice were kept in their home cages for 4 weeks before being killed for preparation of slices and recording of transfected and non-transfected neurons, identified by the presence or absence of eGFP. The estimated amount of cells transfected varied from experiments to experiments and was ~15 cells per slice and 600 cells per infection (similar profile to Supplementary Fig. 6).

The expressing RABV were stereotetically injected as previously described\textsuperscript{46} in CA1 stratum radiatum and the cellular CA3 morphology was assessed after 6 days. For STED microscopy, we performed immunohistochemistry (anti-GFP Alexa 488) on 60 μm brain sections to further increase the signal to background ratio.

**Immunohistochemistry.** (a) For immunolabelling performed on RABV infected brains, coronal sections (60 μm) were washed three times with 0.5% Triton-X100 (TX) in 1× PBS (20 min each) and pre-incubated 1 h in a solution (goat serum 10% + 0.5% TX in 1× PBS). The sections were washed three times with 1× PBS and immersed in the primary antibody solution (rabbit anti-GFP 1/5000; Invitrogen; goat anti-diabetic peptide antibody 1/500; ABCAM) overnight, rinsed under TX (1× 5 min) TX (1× 5 min) and washed three times with PBS before incubation overnight with the secondary antibodies (goat anti-rabbit Alexa Fluor 488 antibody, A11008 from Invitrogen; 1/1000 diluted in 1× PBS + 0.5% TX) for 2 h at room temperature. The sections were rinsed in PBS (2× 5 min) and coverslipped using DAKO Fluor 488. (c) Immunohistochemistry to determine the cell types infected in the hippocampal CA3 area was performed on sections from lentivirus-infected mice as described above, using following antibodies: anti-NeuN (mouse Millipore AB107, 1/400), anti-GFAP (rabbit Millipore AB5804, 1/1000), donkey anti-mouse secondary antibody coupled Alexa Fluor 594 (1/200) and donkey anti-rabbit secondary antibody coupled Alexa Fluor 488 (1/200).

**STED microscopy and morphometric analysis.** Super-resolved images of spine morphology were obtained with a custom-built STED microscope with a nominal spatial resolution of 50 nm. All images were acquired in the stratum radiatum above the CA3b region as stacks of 10 z-sections with a step size of 192 nm and a pixel size of 40 nm × 40 nm. For spine counts and density calculations over large field-of-views (image from Fig. 2a left and data Fig. 2b), a home-built two-photon microscope (Chameleon Coherent, Co.; OPO, APE) for fluorescence quenching \( (\lambda_{\text{STED}} = 955 \text{ nm}, \approx 200 \text{ ps}) \), as described previously\textsuperscript{44}. The pulses of originally 200 fs duration were stretched to ~300 ps by dispersion via a 100-m-long
polarization-preserving fiber (Schäfer + Kirchhoff, Hamburg, Germany). To create the STED focal doughnut, a polymeric phase plate (RPC Photonics, Rochester, NY) was introduced into the path of the expanded STED beam, imprinting a helical phase ramp onto the waveform. The STED and excitation pulses were synchronized via external triggering of the laser diode, and the delay was adjusted with a custom-built electronic delay generator. Both beams were overlapped with a dichroic mirror (AHF Analysentechnik, Tübingen, Germany), and focused onto the sample through a custom objective (HCX PL APO, 1.47 NA, Leica, Wetzlar, Germany). A telecentric beam scanner (Yanus IV, TILL Photonics, Gräfelfing, Germany) with scan and tube lenses from the microscope manufacturer was used to steer the beam. Focusing by the objective was controlled via a piezo actuator (P-721 PIFOC, Physik Instrumente, Karlsruhe, Germany). The fluorescence was collected episcopically using a dichroic mirror and a 525/50 band-pass filter, and imaged onto a multimode optical fiber connected to an avalanche photodiode (SPCM-AQR-13-FC, PerkinElmer, Waltham, MA). Images were acquired using ImSpector software (courtesy of A. Schöne, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). All morphological measurements were done using a custom-made plug-in for ImageJ where the spine length was measured from the base of the dendrite to the edge of the head, following the curvature of the spine neck. Spine neck width is reported as the average from multiple spine neck profiles, drawn orthogonal to the spine neck curvature. Spine head width was measured as the smallest distance along the spine axis. The length and width of each spine neck was determined by measuring the full width at half-maximum of a Gaussian fit applied to line profiles across the spine neck. To evaluate the impact of nanoscale alterations in spine morphology on diffusional coupling, we calculated the compartmentalization factor, which is defined as:

\[ CF = \frac{V + L}{L} \]

where \( V \) is the spine head volume, \( L \) the spine neck length and \( A \) the cross-sectional area of the spine neck. compartmentalization factor corresponds to the time constant \( \tau \) of diffusional recovery \( (\tau = \frac{L}{A}) \) after a step change in concentration of a molecule with a diffusion coefficient \( D \), serving as a quantitative measure of the degree of biochemical compartmentalization of a synapse.

**Binding assays.** On collection, the two hippocampi from each mouse were stored at \(-80^\circ\)C. The hippocampi were then unfrozen in ice-cold Krebs solution (140 mM NaCl, 5 mM KCl, 25 mM HEPES, 1 mM EDTA, 10 mM glucose and pH 7.4) and sliced in a McIlwain chopper (800 μm). Each individual slice was then placed on a rubber surface, over a drop of ice-cold Krebs solution, and CA3 sublices were manually dissected under microscope magnification. To purify synaptic membranes, the CA3 sublices from each animal were placed in an apparatus with 500 μl of ice-cold sucrose solution (0.32 M). Membrane fractionation was performed by centrifugation at 14,000 g for 20 min at 4 °C, and the supernatants were collected, centrifuged at 14,000 g for 20 min at 4 °C and the pellet was resuspended in 500 μl of 45% (v/v) Percoll solution made up in a Krebs solution solution. After centrifugation at 14,000 g for 2 min at 4 °C, the top layer was removed (synaptosomal fraction), washed in 500 μl of Krebs solution and vigorously resuspended in 500 μl of Tris/Mg solution (50 mM Tris and 10 mM MgCl₂, pH 7.4). The mixtures were centrifuged at 30,000 g for 30 min to pull-down synaptic membranes and resuspended in 500 μl of Tris/Mg solution with 4 U/ml aprotinin. To remove contaminating microglia (ONAOV; generously offered by Dr. Ennio Ongini, Shering-Plough, Milan, Italy) or of H-DPCPX (specific activity of 102.1 Ci mmol⁻¹; DuPont NEN, Boston, MA, USA) was for 2 h at room temperature with 6.5–13.5 μg ml⁻¹ of synaptosomal protein in a final volume of 200 μl in Tris/Mg solution containing 4 U ml⁻¹ adenosine deaminase with a single sup (to contain more than two independent groups) was performed; otherwise, a Mann–Whitney test was used to compare two groups) and Kruskal–Wallis test followed by a Dunn’s multiple comparison test (to compare more than two groups). Within-cell comparisons were made with Wilcoxon matched pairs test using non-normalized values. Data distributions were analysed using the Kolmogorov–Smirnov test with the data from wt mice as reference. Statistical differences were considered significant at \( P < 0.05 \).

**Data availability.** The data set that supports the findings of this study are available from the corresponding author on request.

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Author contributions

S.V.S. and P.Z. performed the electrophysiology recordings. S.V.S. performed all the electrophysiology analysis. S.V.S. and M.G.H. performed all stereotaxic injections and subsequent immunolabelings. P.B. and V.N. performed STED acquisitions. P.B. and S.V.S. and P.Z. performed the electrophysiology recordings. S.V.S. performed all the subsequent immunolabellings. P.B. and V.N. performed STED acquisitions. P.B. and S.V.S. and P.Z. performed the electrophysiology recordings. S.V.S. performed all the subsequent immunolabellings. P.B. and V.N. performed STED acquisitions.
experiments. A.G. performed immunostaining anti amyloid-beta plaques. F.Q.G. prepared synaptic membranes. C.B. provided help with electrophysiology analysis. R.A.C. performed binding assays and analysis, and helped with discussion. N.Go. produced shRNA and scrRNA lentiviral constructs and performed expression experiments. M.G.H. and A.F. provided rabies virus. C.M. provided supervision, guided the project and wrote the manuscript together with S.V.S. All authors discussed the results and commented on the manuscript.

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