

Article

Adenosine A_{2A} Receptor Up-Regulation Pre-Dates Deficits of Synaptic Plasticity and of Memory in Mice Exposed to A β _{1–42} to Model Early Alzheimer's Disease

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Abstract: The intracerebroventricular (icv) injection of amyloid peptides (A β) models Alzheimer's disease (AD) in mice, as typified by the onset within 15 days of deficits of memory and of hippocampal long-term potentiation (LTP) that are prevented by the blockade of adenosine A_{2A} receptors (A_{2A}R). Since A_{2A}R overfunction is sufficient to trigger memory deficits, we tested if A_{2A}R were upregulated in hippocampal synapses before the onset of memory deficits to support the hypothesis that A_{2A}R overfunction could be a trigger of AD. Six to eight days after A β -icv injection, mice displayed no alterations of hippocampal dependent memory; however, they presented an increased excitability of hippocampal synapses, a slight increase in LTP magnitude in Schaffer fiber-CA1 pyramid synapses and an increased density of A_{2A}R in hippocampal synapses. A_{2A}R blockade with SCH58261 (50 nM) normalized excitability and LTP in hippocampal slices from mice sacrificed 7–8 days after A β -icv injection. Fifteen days after A β -icv injection, mice displayed evident deficits of hippocampal-dependent memory deterioration, with reduced hippocampal CA1 LTP but no hyperexcitability and a sustained increase in synaptic A_{2A}R, which blockade restored LTP magnitude. This shows that the upregulation of synaptic A_{2A}R precedes the onset of deterioration of memory and of hippocampal synaptic plasticity, supporting the hypothesis that the overfunction of synaptic A_{2A}R could be a trigger of memory deterioration in AD.

Keywords: adenosine; A_{2A} receptor; Alzheimer's disease; memory; LTP; synapse



Citation: Lopes, C.R.; Silva, A.C.; Silva, H.B.; Canas, P.M.; Agostinho, P.; Cunha, R.A.; Lopes, J.P. Adenosine A_{2A} Receptor Up-Regulation Pre-Dates Deficits of Synaptic Plasticity and of Memory in Mice Exposed to A β _{1–42} to Model Early Alzheimer's Disease. *Biomolecules* **2023**, *13*, 1173. <https://doi.org/10.3390/biom13081173>

Academic Editors: Nian Xiong, Tao Wang and Zhentao Zhang

Received: 16 June 2023
Revised: 19 July 2023
Accepted: 26 July 2023
Published: 28 July 2023



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1. Introduction

Alzheimer's disease (AD) is the most common memory pathology. It is neurochemically characterized by an abnormal production of amyloid peptides (A β) and an hyperphosphorylation of microtubule-associated tau protein [1]. One of the earliest alterations in AD evolution is a dysfunction and loss of synapses [2–4]—in particular, of glutamatergic synapses [5,6]—with aberrant patterns of synaptic plasticity in prominently afflicted regions, namely, in the hippocampus (e.g., [7,8]). However, the mechanisms triggering this synaptotoxicity, already present in mild cognitive impairment [9–11], considered a prodrome of AD, are still unknown.

The adenosine modulation system has emerged in recent years as a candidate target to alleviate the burden of AD. Adenosine is released in an activity-dependent manner [12,13] and is responsible for assisting in the encoding of information salience in neuronal networks through a combined action of inhibitory A₁ receptors (A₁R) and facilitatory A_{2A} receptors (A_{2A}R) (reviewed in [14]). Both A₁R and A_{2A}R are mainly located in excitatory synapses in the cerebral and limbic cortices [15–17]. In particular, A_{2A}R increase glutamate release [18,19] and bolster NMDA receptor function in the hippocampus [20–22] to

selectively control synaptic plasticity (e.g., [23,24]) as well as synaptotoxicity (e.g., [24–26]). Notably, the pharmacological, genetic or optogenetic overfunction of A_{2A}R is actually sufficient to deteriorate behavioral performance in tests of spatial reference memory [22,27–29], and, conversely, the pharmacological or genetic blockade of A_{2A}R afford a general neuroprotection against brain damage (reviewed in [14,30,31]) and prevent memory dysfunction in different animal models of AD [8,22,25,32–34]. This is in notable agreement with human epidemiological findings showing that the regular intake of caffeinated coffee (caffeine is a selective antagonist of central adenosine receptors [35]) attenuates memory deficits and the onset of AD (e.g., [36–38]) and that A_{2A}R polymorphisms are associated with the incidence of AD [39,40]. Importantly, in different models of neurodegeneration, A_{2A}R undergo a gain of function, typified by a parallel upregulation of cortical A_{2A}R (e.g., [8,22,24–26,29,33]) and an increased formation of ATP-derived adenosine within cortical synapses [41–45], which is selectively directed at the activation of A_{2A}R [20,41–45]. Furthermore, in animal models of temporal lobe epilepsy [42] and of Parkinson's disease [45], A_{2A}R overfunction predates alterations of synaptic plasticity and of behavior that are characteristic of these diseases, prompting the hypothesis that A_{2A}R upregulation might actually be a trigger of brain dysfunction resulting from aberrant synaptic plasticity [46].

We took advantage of a model of early AD based on the intracerebroventricular (icv) administration of a suspension of soluble β -amyloid peptides monomers and oligomers (A β _{1–42}) to test if hippocampal A_{2A}R upregulation was a precocious event, i.e., pre-dating hippocampal-dependent memory deficits. Previous studies have shown that the selective A_{2A}R antagonist SCH58261 prevents A β -induced dysfunction of synaptic plasticity and of memory [25,41], and that there is time gap of at least 14 days between the icv administration of A β _{1–42} and the establishment of memory dysfunction [25]. This was exploited to test if A_{2A}R density was increased in hippocampal synapses before the onset of memory dysfunction and if this was coupled to adaptive compensatory alterations of synaptic transmission and plasticity in the AD presymptomatic period.

2. Materials and Methods

2.1. Animals

We used adult C57bl/6j mice, 3 months of age (24.2 ± 1.7 g) and of both sexes (20 females and 12 males for the data presented in Figures 1 and 2; 14 females and 18 males in the groups treated with the A_{2A}R antagonist in Figure 3, with similar number of mice from each sex in each of the two groups), obtained from Charles River (Barcelona, Spain). We did not carry out a de novo power analysis, since we relied on our previous experience of the variability associated with the model used and drugs tested to pre-define the sample size as 8 mice per group in the behavioral and electrophysiology experiments and a minimum of 6 mice per group in the binding experiments in order to be able to detect 20% changes with 95% confidence. Mice were housed under controlled temperature (23 ± 1 °C) and a 12 h light/dark cycle, with free access to food and water. Mice were handled following European Community guidelines (EU Directive 2010/63/EU) and the Portuguese law on animal care (1005/92), and all procedures were approved by the Ethical Committee of the Center for Neuroscience and Cell Biology of Coimbra (ORBEA-128/2015).

2.2. Intracerebroventricular Injection of A β to Model AD

The A β _{1–42} peptide fragment was purchased from Bachem (Bubendorf, Germany) and dissolved in water to obtain a solution mostly composed of small-molecular-weight A β oligomers [25,47] with a final concentration of 2.25 mg/mL. Mice were subjected to stereotaxic surgery for unilateral intracerebroventricular (icv) injection alternatively in the right or left hemisphere (dorsoventral: -2.00 mm; anteroposterior: -0.58 mm; lateral: ± 1.13 mm) of A β (single dose of 2 nmol of A β _{1–42} in 4 μ L) or vehicle (water, which caused no behavioral or neurochemical effects, similarly to the administration of scrambled A β _{42–1} (see [25])) under anesthesia with avertin, as previously performed [6,25,45]. This dose of A β _{1–42} translates into levels of 5–30 pmol A β _{1–42} within the hippocampus, causing synaptic

alterations and selective reference memory dysfunction after 14 days without evidence of cellular damage [25], thus constituting a model of early AD, which has been previously used by different groups (e.g., [25,48,49]).

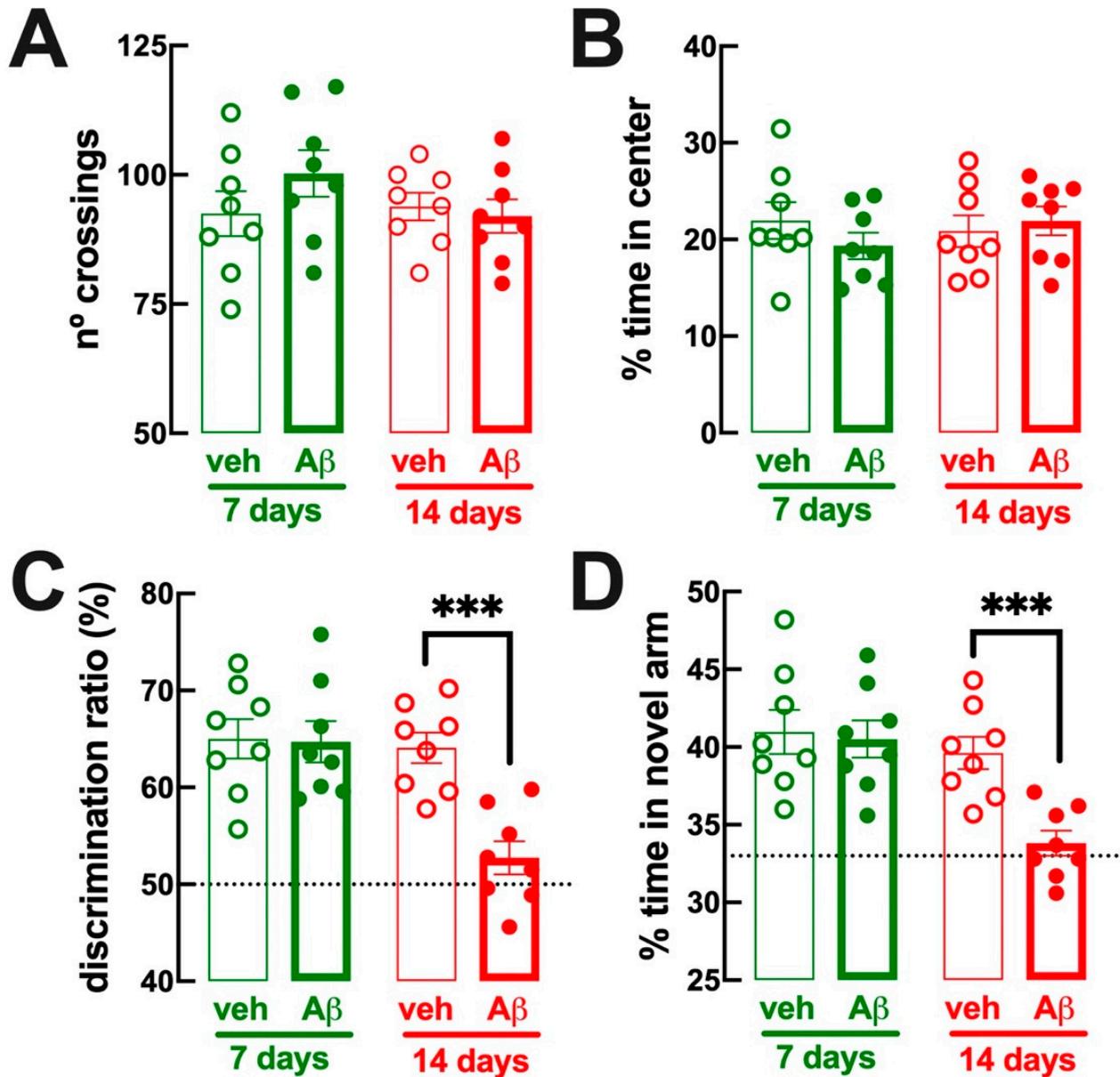


Figure 1. Mice challenged with an intracerebroventricular (icv) injection of β -amyloid (1–42) (A β) displayed a selective alteration of hippocampal-dependent spatial memory after 14–15 days, without behavioral alterations after 6–7 days. Compared to vehicle-icv-injected mice (veh, thin bars and open symbols), A β -icv-injected mice (A β , thick bars and filled symbols) analyzed at 6–7 days (green bars and symbols) or 14–15 days (red bars and symbols) after icv injections displayed a preserved spontaneous locomotion (A) and a preserved pattern of anxiety (B) in an open field arena. Memory performance in the object displacement test (C) and in the modified Y-maze test (D) was preserved after 6–7 days and deteriorated at 14–15 days. Data are mean \pm SEM; $n = 8$ mice per group; *** $p < 0.001$, Student's t test with Welsh correction.

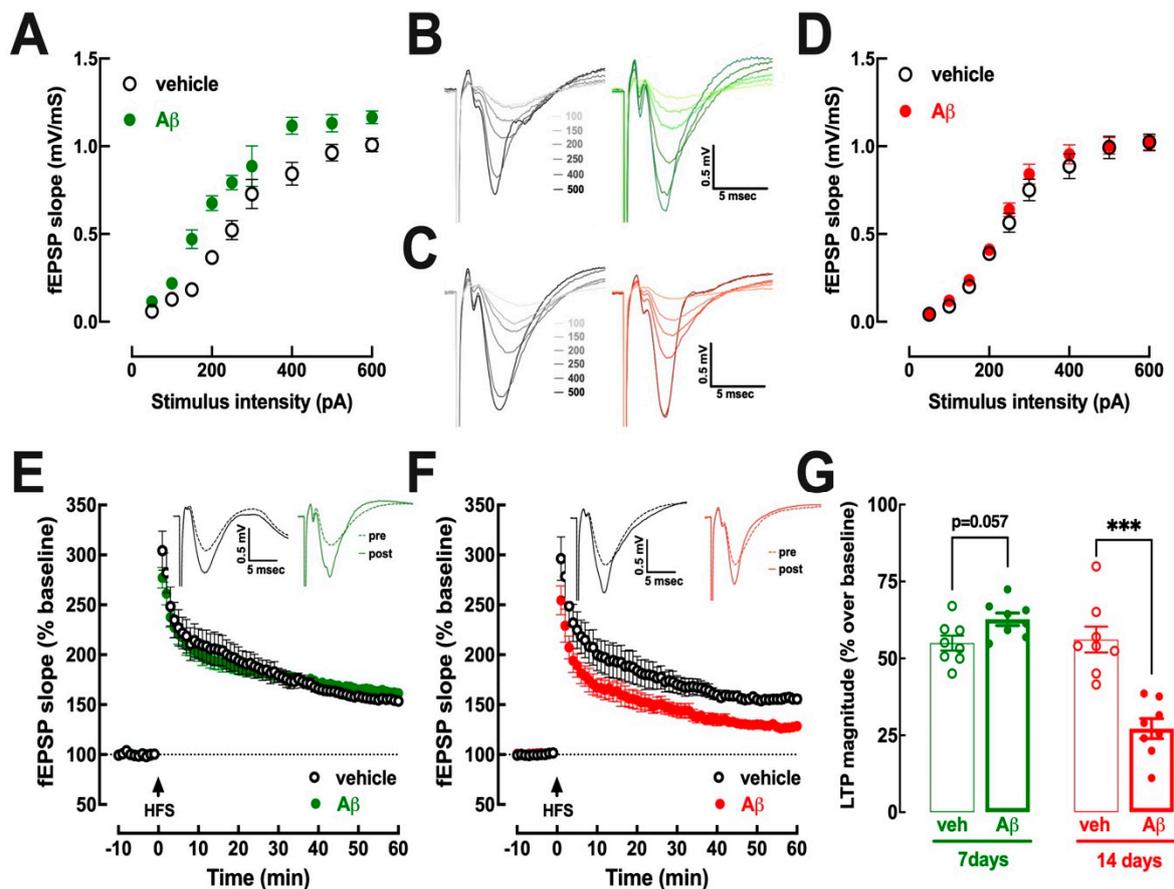


Figure 2. Hippocampal slices collected from mice during the presymptomatic period 7–8 days after an intracerebroventricular (icv) injection of β -amyloid 1–42 ($A\beta$ -icv) displayed an hyperexcitability that was converted to a decreased pattern of synaptic plasticity upon onset of memory deficits at 15–16 days after $A\beta$ -icv. At 7–8 days after $A\beta$ -icv, and when compared to vehicle-icv injected mice (black open symbols/lines), $A\beta$ -icv mice (filled green symbols/lines) displayed an hyperexcitability assessed by the shift to left of the input/output curve of the field excitatory post-synaptic potentials (fEPSP) slope (A,B) and a tendency towards an increased magnitude of long-term potentiation (LTP) triggered by a high-frequency stimulation train (HFS, 100 Hz for 1 s) in $A\beta$ -icv mice (E,G). In contrast, in the symptomatic phase, there was no difference in the input/output curve between control and $A\beta$ -icv mice (filled red symbols/lines) (C,D); however, LTP magnitude was lower in $A\beta$ -icv mice (F,G). Data are mean \pm SEM of 8 mice per group, the same as in Figure 1; *** $p < 0.001$ between indicated groups using Student's t test with Welch correction.

Behavioral analysis was performed before the onset of memory deficits (6–7 days after $A\beta$ -icv injection) or when memory deficits became evident (14–15 days after $A\beta$ -icv). Mice in the two groups were sacrificed in pairs (1 vehicle- and 1 $A\beta$ -treated) between days 7–8 or days 15–16 after $A\beta$ -icv injections by decapitation after deep anesthesia with 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane from Sigma-Aldrich, Lisbon, Portugal; no reaction to handling or tail pinch, while still breathing).

2.3. Behavioral Analysis

All behavior tests were carried out as previously described [8,24,33,41] from 9 AM until 5 PM by experimenters who were unaware of drug treatments in a sound-attenuated room maintained at 21–23 $^{\circ}$ C and 50–60% humidity with red lightening (8 lux light intensity) and visual cues on the walls, to which the animals were previously habituated for at least 1 h before beginning behavioral tests. The tests were video-recorded and analyzed with the

ANY-maze Video Tracking Software (version 6.18; Stoelting Europe, Dublin, Ireland). The apparatuses were cleaned with 10% ethyl alcohol to remove odors after testing each mouse.

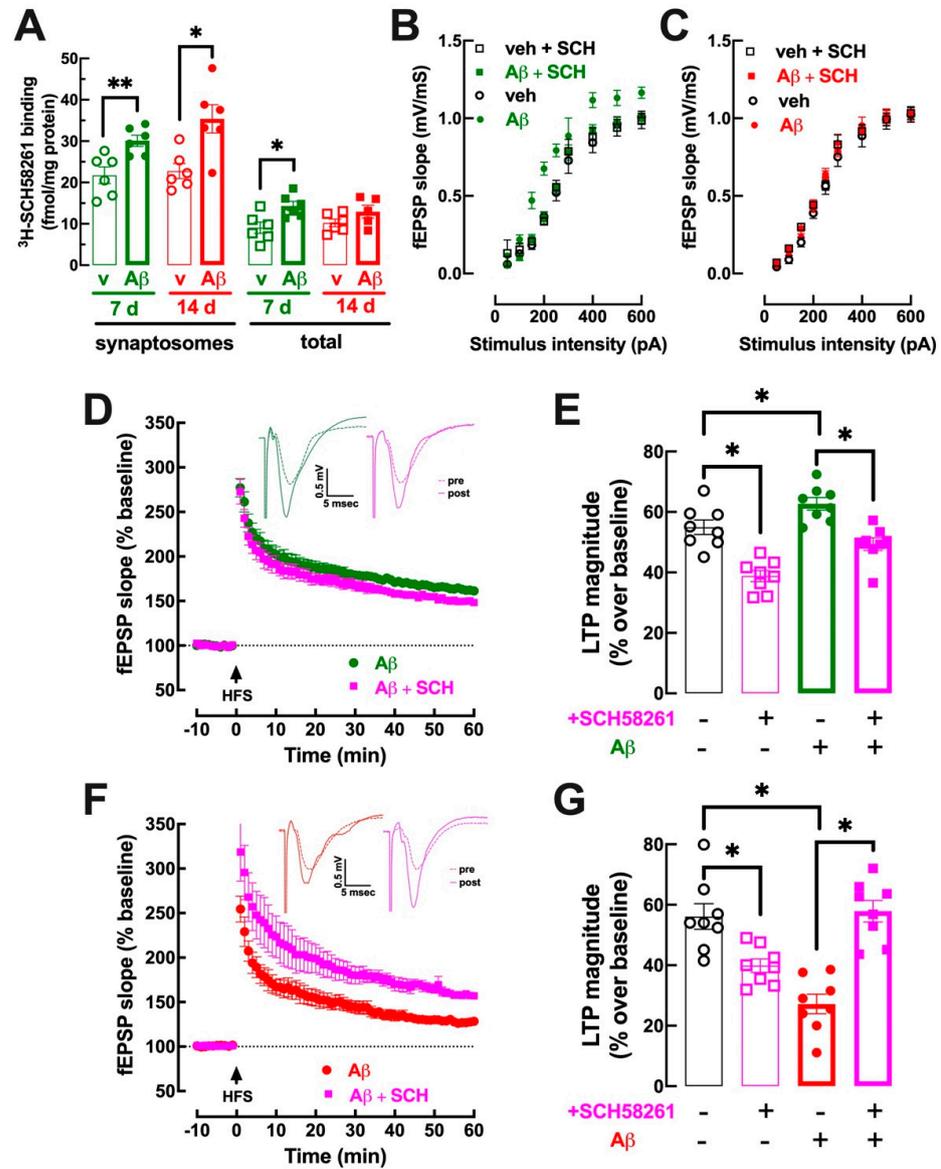


Figure 3. A_{2A}R are upregulated from the presymptomatic phase of AD onward and control both hippocampal hyperexcitability in the presymptomatic phase and LTP deficits upon onset of memory deficits. (A) Binding of the A_{2A}R antagonist ³H-SCH58261 (2 nM) in vehicle-icv mice (v, open symbols and thin bars) and in A β -icv mice (A β , filled symbols and thick bars) in hippocampal synaptosomal or total membranes at 7–8 days (green) or 15–16 days (red) after icv injections. Data are mean \pm SEM of 6 mice per group; * $p < 0.05$ and ** $p < 0.01$ between indicated groups using Student’s *t* test with Welch correction. (B) The addition of SCH58261 (50 nM) to hippocampal slices prevented the hyperexcitability in A β -icv mice and had no effect in vehicle-icv mice (veh) at 7–8 days, whereas SCH58261 was devoid of effects on hippocampal excitability in A β -icv and vehicle-icv mice at 15–16 days (C). SCH58261 decreased LTP magnitude triggered by a high-frequency stimulation train (HFS, 100 Hz for 1 s) in vehicle-icv-treated mice and normalized LTP magnitude in A β -icv both in the presymptomatic (D,E) and symptomatic phase (F,G). The vehicle-icv and A β -icv groups in Figure 3 are the same as in Figure 2. Data in (B–G) are mean \pm SEM of 8 mice per group; * $p < 0.05$ between indicated groups using Tukey’s test after a two-way ANOVA.

Locomotion and exploratory behavior were monitored in the morning of either day 6 or day 14 after A β -icv injections, using an open-field arena, where each mouse was placed in the center of the open field to record during 10 min the following variables: number of peripheral squares (adjacent to the walls) crossed (peripheral locomotion); number of central squares (away from the walls) crossed (central locomotion); and total locomotion (peripheral locomotion plus central locomotion).

Hippocampal-dependent memory was evaluated using the object displacement test, carried out in the afternoon of either day 6 or day 14 after icv injections. Mice were exposed to two identical objects in the same open field apparatus in which they were habituated and were allowed to explore for 5 min the objects fixed in opposite corners 5 cm away from walls and 25 cm apart from each other. In a test trial carried out 2-h later, mice were again placed for 5 min in the open field arena, except that one of the objects was moved to a novel position. Memory performance was quantified with an object displacement index defined as the ratio between the time exploring the object in the novel location over the total time exploring both objects. Exploration of an object is defined as directing the nose to the object at a distance equal to or less than 1 cm from the object and/or touching it with the nose; rearing on to object was not considered exploratory behavior.

Spatial memory was further evaluated using a 2-trials Y-maze paradigm on the morning of either day 7 or day 15 after icv injections. The test consisted of two sessions of 8 min duration separated by a 90-min inter-trial interval. During the first session, the mouse was placed at the end of one arm and allowed to explore the two available arms since the third arm (the novel arm) was blocked by a guillotine door. During the second session, the 'novel' arm was open, and the mouse was placed in the start arm and allowed to explore the three arms. Memory performance was evaluated by measuring the time spent exploring the 'novel' arm compared to the exploration of the other two arms. An entry into an arm was defined as placement of all four paws into the arm.

2.4. Electrophysiological Recordings

Following sacrifice, the mouse brain was quickly removed and placed in ice-cold, oxygenated (95% O₂, 5% CO₂), artificial cerebrospinal fluid (ACSF; in mM: 124.0 NaCl, 4.4 KCl, 1.0 Na₂HPO₄, 25.0 NaHCO₃, 2.0 CaCl₂, 1.0 MgCl₂, 10.0 glucose). One hemisphere was used for electrophysiological experiments and the other hemisphere was used for receptor binding studies (in 6 out of the 8 mice per group). Slices (400 μ m-thick) from the dorsal hippocampus were cut transverse to the long axis of the hippocampus with a McIlwain tissue chopper (Mickle Laboratory Engineering Co., Surrey, UK) and maintained for at least 1 h prior to recording in a holding chamber with oxygenated ACSF at room temperature. Slices were then transferred to a submerged recording chamber and superfused at 3 mL/min with oxygenated ACSF kept at 30.5 °C. Electrophysiological recordings of field excitatory postsynaptic potentials (fEPSP) were carried out as previously described (e.g., [24,33,35,41]) with the recording electrode, filled with 4 M NaCl (2–5 M Ω resistance), placed in the CA1 stratum radiatum targeting the distal dendrites of pyramidal neurons and the stimulating bipolar concentric electrode placed in the proximal CA1 stratum radiatum. Rectangular pulses of 0.1 ms were delivered every 20 s through a Grass S44 or a Grass S48 square pulse stimulator (Grass Technologies, Singapore). After amplification (ISO-80, World Precision Instruments, Singapore), the recordings were digitized (Pico ADC-42, Pico Technologies Ltd., St. Neots, UK), averaged in groups of 3 and analyzed using the WinLTP version 2.10 software [50].

Input/output (I/O) curves, in which the percentage of the maximum fEPSP slope was plotted versus stimulus intensity, were first performed in all slices to evaluate changes in basal synaptic transmission and to determine the adequate level of electrical stimulation required to trigger between 50–60% of maximal fEPSP response, which was used for the remainder of the experimental protocol. Long-term potentiation (LTP) was induced by a high-frequency stimulation train (100 Hz for 1 s). LTP was quantified as the percentage change between the average slope of the 10 fEPSPs between 50 and 60 min after LTP

induction in relation to the average slope of the fEPSPs measured during the 10 min that preceded LTP induction. The effect of SCH58261 (50 nM) on LTP was assessed by comparing LTP magnitude in the absence and presence of SCH58261 in experiments carried out in different slices from the same animal.

2.5. Membrane Binding Assays

Following sacrifice, one hippocampus of each mouse was dissected and homogenized in ice-cold sucrose solution (0.32 M D-sucrose; 1 mM EDTA-Na; 10 mM HEPES; 0.015 mM bovine serum albumin; pH 7.4 at 4 °C) for subsequent preparation of synaptosomes and total membranes, as previously described (e.g., [16,17]). The synaptosomal fraction and the total membrane fraction were each resuspended in 300 µL of a pre-incubation solution (50 mM Tris, 1 mM EDTA, 2 mM EGTA, pH 7.4) to determine their protein content with the Bio-Rad protein assay (Bio-Rad, Amadora, Portugal) and stored at −80 °C until used for membrane binding assays.

Binding assays to estimate A_{2A}R density in synaptosomal membranes were carried out as previously described (e.g., [8,24,26,33]). Briefly, the synaptosomes were lysed in a Tris/Mg solution (50 mM Tris, 10 mM MgCl₂, pH 7.4) and pelleted. Both synaptosomal membranes and total membranes were incubated for 30 min at 37 °C with adenosine deaminase (2 U/mL; Roche Molecular Biochemicals, Indianapolis, IN, USA) to remove endogenous adenosine. After centrifugation at 24,000 × *g* for 15 min at 4 °C, the pellets were resuspended in 600 µL of Tris/Mg solution with 4 U/mL of adenosine deaminase. A_{2A}R binding density was determined with 217–345 µg of synaptosomal membrane protein exposed during 1 h at room temperature to a single supra-maximal concentration (2 nM) of the selective antagonist ³H-SCH58261 (specific activity of 77 Ci/mmol, prepared by GE Healthcare Life Sciences and generously offered by E. Ongini, Schering-Plough, Italy) in a final volume of 200 µL. The binding reactions were stopped by addition of 5 mL of ice-cold Tris/Mg and vacuum filtration through glass fiber filters (Whatman GF/C filters, GE Healthcare Life Sciences, Carnaxide, Portugal), followed by a washing step with 5 mL of ice-cold Tris/Mg. Filters were then placed in vials with 2 mL of scintillation liquid (AquaSafe 500Plus; Zinsser Analytic GMBH, Eschborn, Germany) to measure radioactivity in a 2900TR Tricarb β-counter (PerkinElmer, Lisbon, Portugal) after at least 12 h. Specific binding was expressed as fmol/mg of protein and determined via subtraction of the non-specific binding, measured in the presence of the mixed A₁/A_{2A} receptor antagonist XAC (Sigma-Aldrich, Lisbon, Portugal) at a concentration (12 µM) over 1000 times higher than that of the radioligand. Total binding measurements were conducted in triplicate, and nonspecific binding in duplicate. Negative controls in hippocampal membranes of A_{2A}R knockout mice have previously ensured the selectivity of the tested concentration of ³H-SCH58261 [51].

2.6. Statistical Analyses

The values are presented as mean ± S.E.M. with the number of determinations (*n*, preparations from different mice). A Grubbs' test was first used to detect putative outliers. The comparison of two experimental conditions was performed using a two-tail Student's *t* test with Welsh correction. Otherwise, statistical analysis was performed by two-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison post hoc test. *p* < 0.05 was considered to represent statistical significance. Statistical analysis was performed using GraphPad Prism software (version 6.0; GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Memory Deficits Are Present at 14 Days but Not at 7 Days after Aβ Administration

The Aβ-icv mouse model of AD is characterized by selective hippocampal-dependent memory deficits emerging 12 days after Aβ-icv administration without motor or emotional alterations [25,41]. Accordingly, compared to vehicle-treated mice, we now observed that 6–7 days after Aβ-icv administration, mice were devoid of alteration of (i) spontaneous

locomotion in the open field test (number of line crossing: 92.5 ± 4.3 for vehicle and 100.3 ± 4.5 for A β -icv, $n = 8$, $p = 0.236$ with Student's t test (Figure 1A)); (ii) emotional-like behavior assessed as the percentage of time spent in the more aversive central zone of the open field arena ($22.0 \pm 1.9\%$ for vehicle and $19.3 \pm 1.4\%$ for A β -icv, $n = 8$, $p = 0.280$ with Student's t test (Figure 1B)); (iii) hippocampal-dependent memory performance in the object displacement test (discrimination ratio: 65.0 ± 2.0 for vehicle and 64.7 ± 2.1 for A β -icv, $n = 8$, $p = 0.915$ with Student's t test (Figure 1C)); and (iv) memory performance in the modified Y-maze test (percentage time in the new arm: $41.0 \pm 1.4\%$ for vehicle and $40.5 \pm 1.2\%$ for A β -icv, $n = 8$, $p = 0.806$ with Student's t test (Figure 1D)). In contrast, 14–15 days after A β -icv administration, mice displayed a selective deficit of hippocampal-dependent memory performance both in the object displacement test (discrimination ratio: 64.1 ± 1.6 for vehicle and 52.7 ± 1.7 for A β -icv, $n = 8$, $p < 0.001$ with Student's t test (Figure 1C); with similar discrimination ratio during the training period: 50.7 ± 1.0 for vehicle and 50.3 ± 1.0 for A β -icv, $n = 8$, $p = 0.778$ with Student's t test), as well as in the modified Y-maze test (percentage time in the new arm: $39.6 \pm 1.0\%$ for vehicle and $33.8 \pm 0.8\%$ for A β -icv, $n = 8$, $p < 0.001$ with Student's t test (Figure 1D)), without evident alterations of locomotion (number of line crossings: 93.9 ± 2.7 for vehicle and 92.0 ± 3.3 for A β -icv, $n = 8$, $p = 0.663$ with Student's t test (Figure 1A)) or anxiety in the open field test (percentage time in the center: $20.9 \pm 1.6\%$ for vehicle and $21.9 \pm 1.5\%$ for A β -icv, $n = 8$, $p = 0.638$ with Student's t test (Figure 1B)). Thus, A β -icv administration only triggers a selective hippocampal-dependent memory deficit at 14–15 days, without evident behavioral modification 6–7 days after A β -icv administration.

3.2. Hippocampal Excitability Increases at 7–8 Days and Decreases at 15–16 Days after A β Administration

In spite of the absence of behavioral alteration at days 6–7 after A β -icv, there were alterations of synaptic excitability in hippocampal slices of these mice. Thus, the recording of synaptic transmission in Schaffer fibers-CA1 pyramid synapses, assessed as field excitatory synaptic potentials (fEPSP), revealed an increase in the input/output (I/O) curves in slices of mice at days 7–8 after A β -icv compared to vehicle-treated controls (Figure 2A,B). This increased excitability is likely to be a transient compensatory feature since it disappeared in slices of mice at days 15–16 after A β -icv, which displayed I/O superimposable to these of vehicle-treated mice (Figure 2C,D).

As shown in Figure 2E,G, synaptic plasticity, assessed as the magnitude of long-term potentiation (LTP), displayed a tendency for an increase ($p = 0.057$, Student's t test) in slices collected at days 7–8 after A β -icv ($62.7 \pm 2.4\%$ over baseline, $n = 8$) compared to vehicle-treated controls ($55.0 \pm 2.4\%$ over baseline, $n = 8$). This tendency for an increased LTP magnitude observed before the onset of memory deficits reverted to a decreased LTP magnitude at the onset of memory deficits (Figure 2F,G), as previously reported [41]. In fact, in slices collected at days 15–16 after A β -icv, LTP magnitude ($27.2 \pm 3.2\%$ over baseline, $n = 8$) was lower ($p < 0.001$, Student's t test) than LTP magnitude in slices from vehicle-treated mice ($56.1 \pm 4.2\%$ over baseline, $n = 8$).

3.3. Early Upregulation of Synaptic A $_{2A}$ R Is Responsible for Abnormal Hippocampal Function at 7–8 Days and at 15–16 Days after A β Administration

Since A $_{2A}$ R activation mimics [22,27,28] and A $_{2A}$ R blockade with SCH58261 prevents A β -icv-induced synaptic and memory deficits [25,41,52], and A $_{2A}$ R are up-regulated before the onset of some brain-induced dysfunction [26,45], we then tested if the density of A $_{2A}$ R in hippocampal synapses increased before the onset of synaptic and memory deficits triggered by A β -icv. As shown in Figure 3A, the binding density of a supramaximal but selective concentration (2 nM) of the A $_{2A}$ R antagonist ^3H -SCH58261 [51] was larger ($p < 0.008$, Student's t test) in hippocampal synaptosomal (i.e., synaptic) membranes from A β -icv-treated mice (30.1 ± 1.4 fmol/mg protein, $n = 6$) than from vehicle-treated mice at 7–8 days (21.8 ± 2.0 fmol/mg protein, $n = 6$), i.e., when memory performance was not yet modified (Figure 1C,D). In accordance with the previously described enrichment of A $_{2A}$ R

in hippocampal synapses [17], the binding density of $A_{2A}R$ in synaptosomal membranes was higher ($p = 0.006$, Student's t test) than in total membranes (9.1 ± 1.4 fmol/mg protein, $n = 6$) (Figure 3A, comparing first to fifth column from the left). However, still, in total hippocampal membranes, $A_{2A}R$ density was higher ($p = 0.015$, Student's t test) 7–8 days after $A\beta$ -icv administration 14.3 ± 1.1 fmol/mg protein, $n = 6$) compared to vehicle-treated mice (9.1 ± 1.4 fmol/mg protein, $n = 6$) (Figure 3A). When $A\beta$ -induced memory deficits became evident, 15 days after $A\beta$ -icv, $A_{2A}R$ density was still larger ($p = 0.012$, Student's t test) in synaptosomal membranes from $A\beta$ -icv treated (35.4 ± 3.4 fmol/mg protein, $n = 6$) compared to vehicle-treated mice (22.8 ± 1.8 fmol/mg protein, $n = 6$) (Figure 3A), but there was no significant difference ($p = 0.175$, Student's t test) between the binding density of $A_{2A}R$ in total membranes from $A\beta$ -icv-treated (13.0 ± 1.5 fmol/mg protein, $n = 5$) compared to vehicle-treated mice (10.2 ± 0.9 fmol/mg protein, $n = 6$) (Figure 3A).

We next investigated if $A_{2A}R$ blockade would be able to prevent the hyperexcitability observed in the hippocampus in the presymptomatic period, i.e., 7–8 days after $A\beta$ -icv injection. The administration of a supramaximal concentration of SCH58261 (50 nM) [20] prevented the increased synaptic efficiency in the hippocampus 7–8 days after $A\beta$ -icv administration, bringing the I/O curve to a profile nearly superimposable to that observed in vehicle-treated mice (Figure 3B); note that the vehicle-icv and $A\beta$ -icv groups in Figure 3B are the same as in Figure 2A). Moreover, the tendency for an increased LTP magnitude observed in hippocampal slices collected 7–8 days after $A\beta$ -icv administration was eliminated by 50 nM SCH58261 (Figure 3D); note that the vehicle-icv and $A\beta$ -icv groups in Figure 3D,E are the same as in Figure 2C–E); thus, in slices collected 7–8 days after $A\beta$ -icv administration, LTP magnitude was $62.7 \pm 2.4\%$ over baseline ($n = 8$) in the absence and was significantly lower ($p < 0.001$, Student's t test) in the presence of SCH58261 ($49.4 \pm 2.1\%$ over baseline, $n = 8$). A Tukey's multiple comparison test after a two-way ANOVA (effect of $A\beta$: $F_{1,28} = 18.82$, $p < 0.001$; effect of SCH58261: $F_{1,28} = 48.61$, $p < 0.001$) revealed no significant difference between LTP magnitude in vehicle-treated and $A\beta$ -icv-treated mice in the presence of SCH58261 ($p = 0.291$), although SCH58261 also decreased LTP magnitude in saline-treated mice ($p < 0.001$, Student's t test), as previously reported (e.g., [26,35,41]).

The normalizing effect of SCH58261 in slices collected 15–16 days after $A\beta$ -icv was similar to that previously described [41,53]. Thus, SCH58261 (50 nM) reverted ($p < 0.001$, Student's t test) the $A\beta$ -induced decrease in LTP magnitude ($27.24 \pm 3.2\%$ over baseline, $n = 8$, in the absence and $57.9 \pm 3.6\%$ over baseline, $n = 8$, in the presence of SCH58261) (Figure 3F,G); note that the vehicle-icv and $A\beta$ -icv groups in Figure 3F,G are the same as in Figure 2D,E). A Tukey's multiple comparison test after a two-way ANOVA (effect of $A\beta$: $F_{1,28} = 6.61$, $p = 0.018$; effect of SCH58261: $F_{1,28} = 4.52$, $p = 0.043$) revealed no significant differences of LTP magnitude between vehicle- and $A\beta$ -icv-treated mice in the presence of SCH58261 ($p = 0.983$), although SCH58261 also decreased LTP magnitude in saline-treated mice ($p = 0.011$), as previously reported (e.g., [26,35,41]).

4. Discussion

The present study shows that an increased density and functional impact of $A_{2A}R$ in the hippocampus predates the $A\beta$ -icv-induced deterioration of synaptic plasticity and of reference memory that are characteristic of this mouse model of AD. Thus, it was shown that the activity of these $A_{2A}R$ —with increased density in the presymptomatic period of AD that is maintained throughout the phenotypic expression of synaptic and memory deficits characteristic of early AD—is required both for the adaptive hyperexcitability before memory deficits as well as for the expression of synaptic and memory deficits present at the onset of symptomatic AD.

We first characterized experimental conditions indicative of a presymptomatic period of AD, where an increase in hippocampal excitability was observed at 7–8 days after $A\beta$ -icv, without any evident alteration of memory-related behavioral performance. This is in agreement with several studies reporting an initial hyperexcitability in the hippocampus in early AD and pre-dating the onset of memory deficits (reviewed in [54–56]), as well as

with the presence of subclinical epileptiform activity in the AD prodrome [57,58] and its association with the onset of AD [59]. This initial hippocampal hyperexcitability is linked to a deregulation of the inhibitory GABAergic network [60–62], involving a parallel alteration of the excitability of different interneurons [60,61,63] as well as an altered astrocytic support [64,65]. Importantly, this initial hippocampal hyperexcitability may contribute to the subsequent deterioration of information processing in hippocampal networks and the onset/aggravation of memory symptoms of AD [54–56], as heralded by the ability of drugs refraining from excessive excitation, such as levetiracetam, to improve performance on spatial memory tasks in early AD [66].

The exploitation of this tentative mouse model of presymptomatic AD then allowed us to conclude that A_{2A}R undergo an early upregulation and seem to be paramount to controlling the increased excitability during this prodrome-like period in this A β -icv model of AD. In fact, we observed an increased density of A_{2A}R in the synapses, where cortical A_{2A}R are most abundant [17], but also in total membranes that mostly include non-synaptic and astrocytic membranes. Moreover, we showed that a selective A_{2A}R antagonist, SCH58261 [51], abrogated the hippocampal hyperexcitability present before the onset of memory deficits in this A β -icv model of AD. Although the precise mechanism underlying hippocampal hyperexcitability in the pathogenesis of AD is still unclear, it has been proposed to involve a deregulation of GABAergic interneurons [60,61,63] and an altered astrocytic support [64,65]. Remarkably, hippocampal A_{2A}R can control the excitability of GABAergic neurons [67], the release of GABA [68,69] and the stability of GABAergic synapses [70]. A_{2A}R are also located in astrocytes in the hippocampus (e.g., [71–73]), controlling GABA uptake [74], and astrocytic A_{2A}R control neuronal excitability and memory performance [73,75,76]. These mechanistic links are in agreement with the observed upregulation of A_{2A}R in synapses as well as in total membranes of the hippocampus during the presymptomatic phase of AD; however, future work is required to unveil the relative contribution of these different mechanism(s) operated by A_{2A}R to control hippocampal hyperexcitability present during the presymptomatic phase of AD.

The pattern of alteration of hippocampal synaptic plasticity in our tentative presymptomatic A β -icv model of AD is also in agreement with the currently accepted evolution of hippocampal activity during the evolution of AD, starting with a hyperexcitability in the presymptomatic phases that gradually evolves into a hypofunction with the onset and aggravation of memory deficits [55,77,78]. The density of synaptic A_{2A}R is increased before and remains increased after the onset on synaptic plasticity and memory deficits in this A β -icv model of AD. Furthermore, A_{2A}R blockade reverted the deficits of synaptic plasticity in a manner similar to that previously reported in the normalization of abnormal synaptic plasticity in the hippocampus in different animal models of AD [8,22,32,33,41] and those of other brain diseases [24,26,42,44,79–83]. The effects of A_{2A}R in the control of abnormal synaptic plasticity likely reflect the key role of A_{2A}R in the control of memory dysfunction, as heralded by the previous observations that A_{2A}R activation is sufficient to trigger memory dysfunction [27–29], whereas A_{2A}R blockade dampens memory dysfunction in AD models [8,22,25,32–34,41], specifically in the currently used A β -icv model of AD [25,41,52]. The mechanisms underlying this beneficial effect of A_{2A}R antagonists are probably related to the impact of A_{2A}R on the release of glutamate [18,19], on AMPA [84] and NMDA receptors [20–22,85,86], as well as the ability of A_{2A}R to control astrocytic function [71–76] and microglia [87–90], impacting neuroinflammation (e.g., [91–93]). Future work is needed to clarify the contribution of these different pools of A_{2A}R to controlling these different processes known to impact synaptic plasticity and memory performance in order to begin tackling the mechanisms associated with the ability of A_{2A}R blockade to normalize synaptic plasticity. These will probably involve the engagement of different heteromers containing A_{2A}R (reviewed in [94]) and the coupling of A_{2A}R to different transducing mechanisms, which may change with the evolution of brain diseases and the upregulation of A_{2A}R (reviewed in [14]). Thus, understanding the spatiotemporal dynamics of A_{2A}R signaling in different compartments—which often have opposite effects on synaptic and behavioral

outputs (see [95–98])—will be critical to understand this apparently paradoxical ability of A_{2A}R blockade to prevent aberrant hyperexcitability and normalize synaptic plasticity both when it is increased as well as when it is decreased.

5. Conclusions

It is concluded that A_{2A}R upregulation is an early event in the presymptomatic phase of AD that is maintained throughout the onset of memory deficits. This prompts consideration of the cortical A_{2A}R upregulation as a putative novel biomarker of the risk of developing AD [46], and eventually as a general biomarker of neuropsychiatric diseases [99] in view of the association of A_{2A}R overfunction with different neuropsychiatric diseases [99]. Moreover, the present observation that A_{2A}R blockade prevents both the initial hyperexcitability during the presymptomatic phase of AD and the opposite decreased synaptic plasticity emerging with the onset of memory deficits confirms the prophylactic and therapeutic potential of A_{2A}R antagonists and stresses the need to understand the different signaling mechanisms operated by A_{2A}R in different cellular compartments.

Author Contributions: C.R.L., A.C.S., P.M.C. and J.P.L. carried out the surgeries and behavioral characterization, C.R.L., A.C.S., H.B.S. and J.P.L. carried out the electrophysiological recordings, A.C.S., P.A. and R.A.C. carried out the neurochemical assays, R.A.C. and P.A. coordinated the project and wrote the manuscript, which was revised and commented by all authors. All authors have read and agreed to the published version of the manuscript.

Funding: Supported by La Caixa Foundation (LCF/PR/HP17/52190001), Centro 2020 (CENTRO-01-0145-FEDER-000008:BrainHealth2020 and CENTRO-01-0246-FEDER-000010) and FCT (POCI-01-0145-FEDER-03127 and UIDB/04539/2020). Cátia R. Lopes is under receipt of a PhD grant from FCT (2021.06954.BD).

Institutional Review Board Statement: All animal procedures were approved by the Ethical Committee of the Center for Neuroscience and Cell Biology of Coimbra (ORBEA-128/2015).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: R.A.C. is a scientific consultant for the Institute for Scientific Information on Coffee. All other authors declare no conflicts of interest.

Abbreviations

A_{2A}R: adenosine A_{2A} receptors; Aβ: amyloid β peptides; ACSF: artificial cerebrospinal fluid; AD: Alzheimer's disease; EPSP: excitatory post-synaptic potential; icv: intracerebroventricular; LTP: long-term potentiation; SCH58261: 2-(2-furanyl)-7-(2-phenylethyl)-7H-pyrazolo [4,3-e][1,2,4]triazolo-[1,5-c]pyrimidin-5-amine.

References

1. Scheltens, P.; De Strooper, B.; Kivipelto, M.; Holstege, H.; Chételat, G.; Teunissen, C.E.; Cummings, J.; van der Flier, W.M. Alzheimer's disease. *Lancet* **2021**, *397*, 1577–1590. [[CrossRef](#)] [[PubMed](#)]
2. Selkoe, D.J. Alzheimer's disease is a synaptic failure. *Science* **2002**, *298*, 789–791. [[CrossRef](#)] [[PubMed](#)]
3. Pelucchi, S.; Gardoni, F.; Di Luca, M.; Marcello, E. Synaptic dysfunction in early phases of Alzheimer's disease. *Handb. Clin. Neurol.* **2022**, *184*, 417–438. [[CrossRef](#)] [[PubMed](#)]
4. Meftah, S.; Gan, J. Alzheimer's disease as a synaptopathy: Evidence for dysfunction of synapses during disease progression. *Front. Synaptic. Neurosci.* **2023**, *15*, 1129036. [[CrossRef](#)]
5. Kirvell, S.L.; Esiri, M.; Francis, P.T. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. *J. Neurochem.* **2006**, *98*, 939–950. [[CrossRef](#)]
6. Canas, P.M.; Simões, A.P.; Rodrigues, R.J.; Cunha, R.A. Predominant loss of glutamatergic terminal markers in a β-amyloid peptide model of Alzheimer's disease. *Neuropharmacology* **2014**, *76*, 51–56. [[CrossRef](#)]

7. Sri, S.; Pegasiou, C.-M.; Cave, C.A.; Hough, K.; Wood, N.; Gomez-Nicola, D.; Deinhardt, K.; Bannerman, D.; Perry, V.H.; Vargas-Caballero, M. Emergence of synaptic and cognitive impairment in a mature-onset APP mouse model of Alzheimer's disease. *Acta Neuropathol. Commun.* **2019**, *7*, 25. [[CrossRef](#)]
8. Viana da Silva, S.; Haberl, M.G.; Zhang, P.; Bethge, P.; Lemos, C.; Gonçalves, N.; Gorlewicz, A.; Malezieux, M.; Gonçalves, F.Q.; Grosjean, N.; et al. Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A_{2A} receptors. *Nat. Commun.* **2016**, *7*, 11915. [[CrossRef](#)]
9. Scheff, S.W.; Price, D.A.; Schmitt, F.A.; Mufson, E.J. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* **2006**, *27*, 1372–1384. [[CrossRef](#)]
10. Galasko, D.; Xiao, M.; Xu, D.; Smirnov, D.; Salmon, D.P.; Dewit, N.; Vanbrabant, J.; Jacobs, D.; Vanderstichele, H.; Vanmechelen, E.; et al. Synaptic biomarkers in CSF aid in diagnosis, correlate with cognition and predict progression in MCI and Alzheimer's disease. *Alzheimer's Dement. Transl. Res. Clin. Interv.* **2019**, *5*, 871–882. [[CrossRef](#)]
11. Zhang, J.; Wang, J.; Xu, X.; You, Z.; Huang, Q.; Huang, Y.; Guo, Q.; Guan, Y.; Zhao, J.; Liu, J.; et al. In vivo synaptic density loss correlates with impaired functional and related structural connectivity in Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **2023**, *43*, 977–988. [[CrossRef](#)]
12. Wall, M.J.; Richardson, M.J.E. Localized adenosine signaling provides fine-tuned negative feedback over a wide dynamic range of neocortical network activities. *J. Neurophysiol.* **2015**, *113*, 871–882. [[CrossRef](#)]
13. Lopes, C.R.; Gonçalves, F.Q.; Olaio, S.; Tomé, A.R.; Cunha, R.A.; Lopes, J.P. Adenosine A_{2A} receptors shut down adenosine A₁ receptor-mediated presynaptic inhibition to promote implementation of hippocampal long-term potentiation. *Biomolecules* **2023**, *13*, 715. [[CrossRef](#)]
14. Cunha, R.A. How does adenosine control neuronal dysfunction and neurodegeneration? *J. Neurochem.* **2016**, *139*, 1019–1055. [[CrossRef](#)]
15. Tetzlaff, W.; Schubert, P.; Kreutzberg, G.W. Synaptic and extrasynaptic localization of adenosine binding sites in the rat hippocampus. *Neuroscience* **1987**, *21*, 869–875. [[CrossRef](#)]
16. Rebola, N.; Pinheiro, P.C.; Oliveira, C.R.; Malva, J.O.; Cunha, R.A. Subcellular localization of adenosine A₁ receptors in nerve terminals and synapses of the rat hippocampus. *Brain Res.* **2003**, *987*, 49–58. [[CrossRef](#)]
17. Rebola, N.; Canas, P.M.; Oliveira, C.R.; Cunha, R.A. Different synaptic and subsynaptic localization of adenosine A_{2A} receptors in the hippocampus and striatum of the rat. *Neuroscience* **2005**, *132*, 893–903. [[CrossRef](#)] [[PubMed](#)]
18. Lopes, L.V.; Cunha, R.A.; Kull, B.; Fredholm, B.B.; Ribeiro, J.A. Adenosine A_{2A} receptor facilitation of hippocampal synaptic transmission is dependent on tonic A₁ receptor inhibition. *Neuroscience* **2002**, *112*, 319–329. [[CrossRef](#)]
19. Machado, N.J.; Simões, A.P.; Silva, H.B.; Ardais, A.P.; Kaster, M.P.; Garção, P.; Rodrigues, D.I.; Pochmann, D.; Santos, A.I.; Araújo, I.M.; et al. Caffeine reverts memory but not mood impairment in a depression-prone mouse strain with up-regulated adenosine A_{2A} receptor in hippocampal glutamate synapses. *Mol. Neurobiol.* **2017**, *54*, 1552–1563. [[CrossRef](#)]
20. Rebola, N.; Lujan, R.; Cunha, R.A.; Mulle, C. Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. *Neuron* **2008**, *57*, 121–134. [[CrossRef](#)]
21. Mouro, F.M.; Rombo, D.M.; Dias, R.B.; Ribeiro, J.A.; Sebastião, A.M. Adenosine A_{2A} receptors facilitate synaptic NMDA currents in CA1 pyramidal neurons. *Br. J. Pharmacol.* **2018**, *175*, 4386–4397. [[CrossRef](#)] [[PubMed](#)]
22. Temido-Ferreira, M.; Ferreira, D.G.; Batalha, V.L.; Marques-Morgado, I.; Coelho, J.E.; Pereira, P.; Gomes, R.; Pinto, A.; Carvalho, S.; Canas, P.M.; et al. Age-related shift in LTD is dependent on neuronal adenosine A_{2A} receptors interplay with mGluR5 and NMDA receptors. *Mol. Psychiatry* **2020**, *25*, 1876–1900. [[CrossRef](#)]
23. Costenla, A.R.; Diógenes, M.J.; Canas, P.M.; Rodrigues, R.J.; Nogueira, C.; Maroco, J.; Agostinho, P.M.; Ribeiro, J.A.; Cunha, R.A.; de Mendonça, A. Enhanced role of adenosine A_{2A} receptors in the modulation of LTP in the rat hippocampus upon ageing. *Eur. J. Neurosci.* **2011**, *34*, 12–21. [[CrossRef](#)] [[PubMed](#)]
24. Kaster, M.P.; Machado, N.J.; Silva, H.B.; Nunes, A.; Ardais, A.P.; Santana, M.; Baqi, Y.; Müller, C.E.; Rodrigues, A.L.S.; Porciúncula, L.O.; et al. Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 7833–7838. [[CrossRef](#)]
25. Canas, P.M.; Porciúncula, L.O.; Cunha, G.M.A.; Silva, C.G.; Machado, N.J.; Oliveira, J.M.A.; Oliveira, C.R.; Cunha, R.A. Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by β -amyloid peptides via p38 mitogen-activated protein kinase pathway. *J. Neurosci.* **2009**, *29*, 14741–14751. [[CrossRef](#)]
26. Canas, P.M.; Porciúncula, L.O.; Simões, A.P.; Augusto, E.; Silva, H.B.; Machado, N.J.; Gonçalves, N.; Alfaro, T.M.; Gonçalves, F.Q.; Araújo, I.M.; et al. Neuronal adenosine A_{2A} receptors are critical mediators of neurodegeneration triggered by convulsions. *eNeuro* **2018**, *5*, ENEURO.0385-18.2018. [[CrossRef](#)]
27. Pagnussat, N.; Almeida, A.S.; Marques, D.M.; Nunes, F.; Chenet, G.C.; Botton, P.H.S.; Mioranza, S.; Loss, C.M.; Cunha, R.A.; Porciúncula, L.O. Adenosine A_{2A} receptors are necessary and sufficient to trigger memory impairment in adult mice. *Br. J. Pharmacol.* **2015**, *172*, 3831–3845. [[CrossRef](#)]
28. Li, P.; Rial, D.; Canas, P.M.; Yoo, J.-H.; Li, W.; Zhou, X.; Wang, Y.; van Westen, G.J.P.; Payen, M.-P.; Augusto, E.; et al. Optogenetic activation of intracellular adenosine A_{2A} receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. *Mol. Psychiatry* **2015**, *20*, 1339–1349. [[CrossRef](#)]

29. Carvalho, K.; Faivre, E.; Pietrowski, M.J.; Marques, X.; Gomez-Murcia, V.; Deleau, A.; Huin, V.; Hansen, J.N.; Kozlov, S.; Danis, C.; et al. Exacerbation of C1q dysregulation, synaptic loss and memory deficits in tau pathology linked to neuronal adenosine A_{2A} receptor. *Brain* **2019**, *142*, 3636–3654. [[CrossRef](#)]
30. Chen, J.-F.; Sonsalla, P.K.; Pedata, F.; Melani, A.; Domenici, M.R.; Popoli, P.; Geiger, J.; Lopes, L.V.; de Mendonça, A. Adenosine A_{2A} receptors and brain injury: Broad spectrum of neuroprotection, multifaceted actions and “fine tuning” modulation. *Prog. Neurobiol.* **2007**, *83*, 310–331. [[CrossRef](#)]
31. Merighi, S.; Borea, P.A.; Varani, K.; Vincenzi, F.; Jacobson, K.A.; Gessi, S. A_{2A} adenosine receptor antagonists in neurodegenerative diseases. *Curr. Med. Chem.* **2022**, *29*, 4138–4151. [[CrossRef](#)] [[PubMed](#)]
32. Laurent, C.; Burnouf, S.; Ferry, B.; Batalha, V.L.; Coelho, J.E.; Baqi, Y.; Malik, E.; Mariciniak, E.; Parrot, S.; Van der Jeugd, A.; et al. A_{2A} adenosine receptor deletion is protective in a mouse model of Tauopathy. *Mol. Psychiatry* **2016**, *21*, 97–107. [[CrossRef](#)] [[PubMed](#)]
33. Silva, A.C.; Lemos, C.; Gonçalves, F.Q.; Pliássova, A.V.; Machado, N.J.; Silva, H.B.; Canas, P.M.; Cunha, R.A.; Lopes, J.P.; Agostinho, P. Blockade of adenosine A_{2A} receptors recovers early deficits of memory and plasticity in the triple transgenic mouse model of Alzheimer’s disease. *Neurobiol. Dis.* **2018**, *117*, 72–81. [[CrossRef](#)] [[PubMed](#)]
34. Orr, A.G.; Lo, I.; Schumacher, H.; Ho, K.; Gill, M.; Guo, W.; Kim, D.H.; Knox, A.; Saito, T.; Saido, T.C.; et al. Istradefylline reduces memory deficits in aging mice with amyloid pathology. *Neurobiol. Dis.* **2018**, *110*, 29–36. [[CrossRef](#)]
35. Lopes, J.P.; Pliássova, A.; Cunha, R.A. The physiological effects of caffeine on synaptic transmission and plasticity in the mouse hippocampus selectively depend on adenosine A₁ and A_{2A} receptors. *Biochem. Pharmacol.* **2019**, *166*, 313–321. [[CrossRef](#)]
36. Eskelinen, M.H.; Ngandu, T.; Tuomilehto, J.; Soininen, H.; Kivipelto, M. Midlife coffee and tea drinking and the risk of late-life dementia: A population-based CAIDE study. *J. Alzheimer’s Dis.* **2009**, *16*, 85–91. [[CrossRef](#)] [[PubMed](#)]
37. Gelber, R.P.; Petrovitch, H.; Masaki, K.H.; Ross, G.W.; White, L.R. Coffee intake in midlife and risk of dementia and its neuropathologic correlates. *J. Alzheimer’s Dis.* **2011**, *23*, 607–615. [[CrossRef](#)] [[PubMed](#)]
38. Dong, X.; Li, S.; Sun, J.; Li, Y.; Zhang, D. Association of coffee, decaffeinated coffee and caffeine intake from coffee with cognitive performance in older adults: National Health and Nutrition Examination Survey (NHANES) 2011–2014. *Nutrients* **2020**, *12*, 840. [[CrossRef](#)]
39. Horgusluoglu-Moloch, E.; Nho, K.; Risacher, S.L.; Kim, S.; Foroud, T.; Shaw, L.M.; Trojanowski, J.Q.; Aisen, P.S.; Petersen, R.C.; Jack, C.R., Jr.; et al. Targeted neurogenesis pathway-based gene analysis identifies ADORA2A associated with hippocampal volume in mild cognitive impairment and Alzheimer’s disease. *Neurobiol. Aging* **2017**, *60*, 92–103. [[CrossRef](#)]
40. Siokas, V.; Moulidou, D.S.; Liampas, I.; Aloizou, A.-M.; Folia, V.; Zoupa, E.; Papadimitriou, A.; Lavdas, E.; Bogdanos, D.P.; Dardiotis, E. Analysis of ADORA2A rs5760423 and CYP1A2 rs762551 genetic variants in patients with Alzheimer’s disease. *Int. J. Mol. Sci.* **2022**, *23*, 14400. [[CrossRef](#)]
41. Gonçalves, F.Q.; Lopes, J.P.; Silva, H.B.; Lemos, C.; Silva, A.C.; Gonçalves, N.; Tomé, Â.R.; Ferreira, S.G.; Canas, P.M.; Rial, D.; et al. Synaptic and memory dysfunction in a β -amyloid model of early Alzheimer’s disease depends on increased formation of ATP-derived extracellular adenosine. *Neurobiol. Dis.* **2019**, *132*, 104570. [[CrossRef](#)] [[PubMed](#)]
42. Augusto, E.; Gonçalves, F.Q.; Real, J.E.; Silva, H.B.; Pochmann, D.; Silva, T.S.; Matos, M.; Gonçalves, N.; Tomé, Â.R.; Chen, J.-F.; et al. Increased ATP release and CD73-mediated adenosine A_{2A} receptor activation mediate convulsion-associated neuronal damage and hippocampal dysfunction. *Neurobiol. Dis.* **2021**, *157*, 105441. [[CrossRef](#)] [[PubMed](#)]
43. Simões, A.P.; Gonçalves, F.Q.; Rial, D.; Ferreira, S.G.; Lopes, J.P.; Canas, P.M.; Cunha, R.A. CD73-mediated formation of extracellular adenosine is responsible for adenosine A_{2A} receptor-mediated control of fear memory and amygdala plasticity. *Int. J. Mol. Sci.* **2022**, *23*, 12826. [[CrossRef](#)] [[PubMed](#)]
44. Dias, L.; Pochmann, D.; Lemos, C.; Silva, H.B.; Real, J.I.; Gonçalves, F.Q.; Rial, D.; Gonçalves, N.; Simões, A.P.; Ferreira, S.G.; et al. Increased synaptic ATP release and CD73-mediated formation of extracellular adenosine in the control of behavioral and electrophysiological modifications caused by chronic stress. *ACS Chem. Neurosci.* **2023**, *14*, 1299–1309. [[CrossRef](#)] [[PubMed](#)]
45. Gonçalves, F.Q.; Matheus, F.C.; Silva, H.B.; Real, J.I.; Rial, D.; Rodrigues, R.J.; Oses, J.-P.; Silva, A.C.; Gonçalves, N.; Prediger, R.D.; et al. Increased ATP release and higher impact of adenosine A_{2A} receptors on corticostriatal plasticity in a rat model of presymptomatic Parkinson’s disease. *Mol. Neurobiol.* **2023**, *60*, 1659–1674. [[CrossRef](#)] [[PubMed](#)]
46. Gessi, S.; Poloni, T.E.; Negro, G.; Varani, K.; Pasquini, S.; Vincenzi, F.; Borea, P.A.; Merighi, S. A_{2A} adenosine receptor as a potential biomarker and a possible therapeutic target in Alzheimer’s disease. *Cells* **2021**, *10*, 2344. [[CrossRef](#)]
47. Resende, R.; Ferreira, E.; Pereira, C.; Resende de Oliveira, C. Neurotoxic effect of oligomeric and fibrillar species of amyloid-beta peptide 1–42: Involvement of endoplasmic reticulum calcium release in oligomer-induced cell death. *Neuroscience* **2008**, *155*, 725–737. [[CrossRef](#)]
48. Hong, S.; Beja-Glasser, V.F.; Nfonoyim, B.M.; Frouin, A.; Li, S.; Ramakrishnan, S.; Merry, K.M.; Shi, Q.; Rosenthal, A.; Barres, B.A.; et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* **2016**, *352*, 712–716. [[CrossRef](#)]
49. Kim, H.Y.; Lee, D.K.; Chung, B.-R.; Kim, H.V.; Kim, Y. Intracerebroventricular injection of amyloid- β peptides in normal mice to acutely induce Alzheimer-like cognitive deficits. *J. Vis. Exp.* **2016**, *109*, 53308. [[CrossRef](#)]
50. Anderson, W.W.; Collingridge, G.L. Capabilities of the WinLTP data acquisition program extending beyond basic LTP experimental functions. *J. Neurosci. Methods* **2007**, *162*, 346–356. [[CrossRef](#)]

51. Lopes, L.V.; Halldner, L.; Rebola, N.; Johansson, B.; Ledent, C.; Chen, J.F.; Fredholm, B.B.; Cunha, R.A. Binding of the prototypical adenosine A_{2A} receptor agonist CGS 21680 to the cerebral cortex of adenosine A₁ and A_{2A} receptor knockout mice. *Br. J. Pharmacol.* **2004**, *141*, 1006–1014. [[CrossRef](#)] [[PubMed](#)]
52. Cunha, G.M.; Canas, P.M.; Melo, C.S.; Hockemeyer, J.; Müller, C.E.; Oliveira, C.R.; Cunha, R.A. Adenosine A_{2A} receptor blockade prevents memory dysfunction caused by β -amyloid peptides but not by scopolamine or MK-801. *Exp. Neurol.* **2008**, *210*, 776–781. [[CrossRef](#)]
53. Lopes, C.R.; Amaral, I.M.; Pereira, M.F.; Lopes, J.P.; Madeira, D.; Canas, P.M.; Cunha, R.A.; Agostinho, P. Impact of blunting astrocyte activity on hippocampal synaptic plasticity in a mouse model of early Alzheimer's disease based on amyloid- β peptide exposure. *J. Neurochem.* **2022**, *160*, 556–567. [[CrossRef](#)]
54. Palop, J.J.; Mucke, L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat. Rev. Neurosci.* **2016**, *17*, 777–792. [[CrossRef](#)] [[PubMed](#)]
55. Targa Dias Anastacio, H.; Matosin, N.; Ooi, L. Neuronal hyperexcitability in Alzheimer's disease: What are the drivers behind this aberrant phenotype? *Transl. Psychiatry* **2022**, *12*, 257. [[CrossRef](#)] [[PubMed](#)]
56. Tang, Y.; Yan, Y.; Mao, J.; Ni, J.; Qing, H. The hippocampus associated GABAergic neural network impairment in early-stage of Alzheimer's disease. *Ageing Res. Rev.* **2023**, *86*, 101865. [[CrossRef](#)]
57. Vossel, K.A.; Beagle, A.J.; Rabinovici, G.D.; Shu, H.; Lee, S.E.; Naasan, G.; Hegde, M.; Cornes, S.B.; Henry, M.L.; Nelson, A.B.; et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol.* **2013**, *70*, 1158–1166. [[CrossRef](#)] [[PubMed](#)]
58. Ranasinghe, K.G.; Kudo, K.; Hinkley, L.; Beagle, A.; Lerner, H.; Mizuiri, D.; Findlay, A.; Miller, B.L.; Kramer, J.H.; Gorno-Tempini, M.L.; et al. Neuronal synchrony abnormalities associated with subclinical epileptiform activity in early-onset Alzheimer's disease. *Brain* **2022**, *145*, 744–753. [[CrossRef](#)]
59. Vossel, K.A.; Ranasinghe, K.G.; Beagle, A.J.; Mizuiri, D.; Honma, S.M.; Dowling, A.F.; Darwish, S.M.; Van Berlo, V.; Barnes, D.E.; Mantle, M.; et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann. Neurol.* **2016**, *80*, 858–870. [[CrossRef](#)]
60. Palop, J.J.; Chin, J.; Roberson, E.D.; Wang, J.; Thwin, M.T.; Bien-Ly, N.; Yoo, J.; Ho, K.O.; Yu, G.-Q.; Kreitzer, A.; et al. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* **2007**, *55*, 697–711. [[CrossRef](#)]
61. Ruiter, M.; Herstel, L.J.; Wierenga, C.J. Reduction of dendritic inhibition in CA1 pyramidal neurons in amyloidosis models of early Alzheimer's disease. *J. Alzheimer's Dis.* **2020**, *78*, 951–964. [[CrossRef](#)] [[PubMed](#)]
62. Li, Y.; Zhu, K.; Li, N.; Wang, X.; Xiao, X.; Li, L.; Li, L.; He, Y.; Zhang, J.; Wo, J.; et al. Reversible GABAergic dysfunction involved in hippocampal hyperactivity predicts early-stage Alzheimer disease in a mouse model. *Alzheimer's Res. Ther.* **2021**, *13*, 114. [[CrossRef](#)] [[PubMed](#)]
63. Wander, C.M.; Li, Y.-D.; Bao, H.; Asrican, B.; Luo, Y.-J.; Sullivan, H.A.; Chao, T.-H.H.; Zhang, W.-T.; Chéry, S.L.; Tart, D.S.; et al. Compensatory remodeling of a septo-hippocampal GABAergic network in the triple transgenic Alzheimer's mouse model. *J. Transl. Med.* **2023**, *21*, 258. [[CrossRef](#)] [[PubMed](#)]
64. Ramos, B.; Baglietto-Vargas, D.; del Rio, J.C.; Moreno-Gonzalez, I.; Santa-Maria, C.; Jimenez, S.; Caballero, C.; Lopez-Tellez, J.F.; Khan, Z.U.; Ruano, D.; et al. Early neuropathology of somatostatin/NPY GABAergic cells in the hippocampus of a PS1xAPP transgenic model of Alzheimer's disease. *Neurobiol. Aging* **2006**, *27*, 1658–1672. [[CrossRef](#)] [[PubMed](#)]
65. Jo, S.; Yarishkin, O.; Hwang, Y.J.; Chun, Y.E.; Park, M.; Woo, D.H.; Bae, J.Y.; Kim, T.; Lee, J.; Chun, H.; et al. GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's disease. *Nat. Med.* **2014**, *20*, 886–896. [[CrossRef](#)] [[PubMed](#)]
66. Vossel, K.; Ranasinghe, K.G.; Beagle, A.J.; La, A.; Ah Pook, K.; Castro, M.; Mizuiri, D.; Honma, S.M.; Venkateswaran, N.; Koestler, M.; et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: A randomized clinical trial. *JAMA Neurol.* **2021**, *78*, 1345–1354. [[CrossRef](#)]
67. Rombo, D.M.; Newton, K.; Nissen, W.; Badurek, S.; Horn, J.M.; Minichiello, L.; Jefferys, J.G.; Sebastiao, A.M.; Lamsa, K.P. Synaptic mechanisms of adenosine A_{2A} receptor-mediated hyperexcitability in the hippocampus. *Hippocampus* **2015**, *25*, 566–580. [[CrossRef](#)]
68. Cunha, R.A.; Ribeiro, J.A. Purinergic modulation of [³H]GABA release from rat hippocampal nerve terminals. *Neuropharmacology* **2000**, *39*, 1156–1167. [[CrossRef](#)] [[PubMed](#)]
69. Saransaari, P.; Oja, S.S. GABA release modified by adenosine receptors in mouse hippocampal slices under normal and ischemic conditions. *Neurochem. Res.* **2005**, *30*, 467–473. [[CrossRef](#)]
70. Gomez-Castro, F.; Zappettini, S.; Pressey, J.C.; Silva, C.G.; Russeau, M.; Gervasi, N.; Figueiredo, M.; Montmasson, C.; Renner, M.; Canas, P.M.; et al. Convergence of adenosine and GABA signaling for synapse stabilization during development. *Science* **2021**, *374*, eabk2055. [[CrossRef](#)]
71. Matos, M.; Augusto, E.; Agostinho, P.; Cunha, R.A.; Chen, J.-F. Antagonistic interaction between adenosine A_{2A} receptors and Na⁺/K⁺-ATPase- α 2 controlling glutamate uptake in astrocytes. *J. Neurosci.* **2013**, *33*, 18492–18502. [[CrossRef](#)] [[PubMed](#)]
72. Orr, A.G.; Hsiao, E.C.; Wang, M.M.; Ho, K.; Kim, D.H.; Wang, X.; Guo, W.; Kang, J.; Yu, G.-Q.; Adame, A.; et al. Astrocytic adenosine receptor A_{2A} and G_s-coupled signaling regulate memory. *Nat. Neurosci.* **2015**, *18*, 423–434. [[CrossRef](#)] [[PubMed](#)]

73. Barros-Barbosa, A.R.; Ferreirinha, F.; Oliveira, Â.; Mendes, M.; Lobo, M.G.; Santos, A.; Rangel, R.; Pelletier, J.; Sévigny, J.; Cordeiro, J.M.; et al. Adenosine A_{2A} receptor and ecto-5'-nucleotidase/CD73 are upregulated in hippocampal astrocytes of human patients with mesial temporal lobe epilepsy (MTLE). *Purinergic Signal.* **2016**, *12*, 719–734. [[CrossRef](#)] [[PubMed](#)]
74. Cristóvão-Ferreira, S.; Navarro, G.; Brugarolas, M.; Pérez-Capote, K.; Vaz, S.H.; Fattorini, G.; Conti, F.; Lluís, C.; Ribeiro, J.A.; McCormick, P.J.; et al. A₁R-A_{2A}R heteromers coupled to G_s and G_{i/0} proteins modulate GABA transport into astrocytes. *Purinergic Signal.* **2013**, *9*, 433–449. [[CrossRef](#)]
75. Matos, M.; Shen, H.-Y.; Augusto, E.; Wang, Y.; Wei, C.J.; Wang, Y.T.; Agostinho, P.; Boison, D.; Cunha, R.A.; Chen, J.-F. Deletion of adenosine A_{2A} receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: Relevance to schizophrenia. *Biol. Psychiatry* **2015**, *78*, 763–774. [[CrossRef](#)]
76. Madeira, D.; Lopes, C.R.; Simões, A.P.; Canas, P.M.; Cunha, R.A.; Agostinho, P. Astrocytic A_{2A} receptors silencing negatively impacts hippocampal synaptic plasticity and memory of adult mice. *Glia* **2023**, *71*, 2137–2153. [[CrossRef](#)]
77. Mufson, E.J.; Mahady, L.; Waters, D.; Counts, S.E.; Perez, S.E.; DeKosky, S.T.; Ginsberg, S.D.; Ikonovic, M.D.; Scheff, S.W.; Binder, L.I. Hippocampal plasticity during the progression of Alzheimer's disease. *Neuroscience* **2015**, *309*, 51–67. [[CrossRef](#)]
78. Baazaoui, N.; Flory, M.; Iqbal, K. Synaptic compensation as a probable cause of prolonged mild cognitive impairment in Alzheimer's disease: Implications from a transgenic mouse model of the disease. *J. Alzheimer's Dis.* **2017**, *56*, 1385–1401. [[CrossRef](#)]
79. Ferreira, D.G.; Batalha, V.L.; Vicente Miranda, H.; Coelho, J.E.; Gomes, R.; Gonçalves, F.Q.; Real, J.I.; Rino, J.; Albino-Teixeira, A.; Cunha, R.A.; et al. Adenosine A_{2A} receptors modulate α -synuclein aggregation and toxicity. *Cereb. Cortex* **2017**, *27*, 718–730. [[CrossRef](#)] [[PubMed](#)]
80. Rei, N.; Rombo, D.M.; Ferreira, M.F.; Baqi, Y.; Müller, C.E.; Ribeiro, J.A.; Sebastião, A.M.; Vaz, S.H. Hippocampal synaptic dysfunction in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis: Reversal by adenosine A_{2A}R blockade. *Neuropharmacology* **2020**, *171*, 108106. [[CrossRef](#)]
81. Moreira-de-Sá, A.; Gonçalves, F.Q.; Lopes, J.P.; Silva, H.B.; Tomé, Â.R.; Cunha, R.A.; Canas, P.M. Adenosine A_{2A} receptors format long-term depression and memory strategies in a mouse model of Angelman syndrome. *Neurobiol. Dis.* **2020**, *146*, 105137. [[CrossRef](#)]
82. Ferrante, A.; Boussadia, Z.; Borreca, A.; Mallozzi, C.; Pedini, G.; Pacini, L.; Pezzola, A.; Armida, M.; Vincenzi, F.; Varani, K.; et al. Adenosine A_{2A} receptor inhibition reduces synaptic and cognitive hippocampal alterations in Fmr1 KO mice. *Transl. Psychiatry* **2021**, *11*, 112. [[CrossRef](#)] [[PubMed](#)]
83. Li, X.-C.; Hong, F.-F.; Tu, Y.-J.; Li, Y.-A.; Ma, C.-Y.; Yu, C.-Y.; Fang, L.; Chen, J.-Y.; Li, Z.-L.; Bao, S.-J.; et al. Blockade of adenosine A_{2A} receptor alleviates cognitive dysfunction after chronic exposure to intermittent hypoxia in mice. *Exp. Neurol.* **2022**, *350*, 113929. [[CrossRef](#)]
84. Dias, R.B.; Ribeiro, J.A.; Sebastião, A.M. Enhancement of AMPA currents and GluR1 membrane expression through PKA-coupled adenosine A_{2A} receptors. *Hippocampus* **2012**, *22*, 276–291. [[CrossRef](#)]
85. Sarantis, K.; Tsiamak, E.; Kouvaros, S.; Papatheodoropoulos, C.; Angelatou, F. Adenosine A_{2A} receptors permit mGluR5-evoked tyrosine phosphorylation of NR2B (Tyr1472) in rat hippocampus: A possible key mechanism in NMDA receptor modulation. *J. Neurochem.* **2015**, *135*, 714–726. [[CrossRef](#)] [[PubMed](#)]
86. Franco, R.; Rivas-Santisteban, R.; Casanovas, M.; Lillo, A.; Saura, C.A.; Navarro, G. Adenosine A_{2A} receptor antagonists affects NMDA glutamate receptor function. Potential to address neurodegeneration in Alzheimer's disease. *Cells* **2020**, *9*, 1075. [[CrossRef](#)] [[PubMed](#)]
87. Orr, A.G.; Orr, A.L.; Li, X.-J.; Gross, R.E.; Traynelis, S.F. Adenosine A_{2A} receptor mediates microglial process retraction. *Nat. Neurosci.* **2009**, *12*, 872–878. [[CrossRef](#)]
88. Gomes, C.; Ferreira, R.; George, J.; Sanches, R.; Rodrigues, D.I.; Gonçalves, N.; Cunha, R.A. Activation of microglial cells triggers a release of brain-derived neurotrophic factor (BDNF) inducing their proliferation in an adenosine A_{2A} receptor-dependent manner: A_{2A} receptor blockade prevents BDNF release and proliferation of microglia. *J. Neuroinflammation* **2013**, *10*, 16. [[CrossRef](#)] [[PubMed](#)]
89. Chen, P.-Z.; He, W.-J.; Zhu, Z.-R.; E, G.-J.; Xu, G.; Chen, D.-W.; Gao, Y.-Q. Adenosine A_{2A} receptor involves in neuroinflammation-mediated cognitive decline through activating microglia under acute hypobaric hypoxia. *Behav. Brain Res.* **2018**, *347*, 99–107. [[CrossRef](#)]
90. Du, H.; Tan, Y.; Li, C.-H.; Zhao, Y.; Li, P.; Ning, Y.-L.; Gao, R.-B.; Wang, B.; Peng, Y.; Tan, S.-W.; et al. High glutamate concentration reverses the inhibitory effect of microglial adenosine 2A receptor on NLRP3 inflammasome assembly and activation. *Neurosci. Lett.* **2022**, *769*, 136431. [[CrossRef](#)] [[PubMed](#)]
91. Rebola, N.; Simões, A.P.; Canas, P.M.; Tomé, A.R.; Andrade, G.M.; Barry, C.E.; Agostinho, P.M.; Lynch, M.A.; Cunha, R.A. Adenosine A_{2A} receptors control neuroinflammation and consequent hippocampal neuronal dysfunction. *J. Neurochem.* **2011**, *117*, 100–111. [[CrossRef](#)] [[PubMed](#)]
92. Meng, F.; Guo, Z.; Hu, Y.; Mai, W.; Zhang, Z.; Zhang, B.; Ge, Q.; Lou, H.; Guo, F.; Chen, J.; et al. CD73-derived adenosine controls inflammation and neurodegeneration by modulating dopamine signalling. *Brain* **2019**, *142*, 700–718. [[CrossRef](#)] [[PubMed](#)]
93. Dias, L.; Lopes, C.R.; Gonçalves, F.Q.; Nunes, A.; Pochmann, D.; Machado, N.J.; Tomé, A.R.; Agostinho, P.; Cunha, R.A. Crosstalk between ATP-P_{2X7} and adenosine A_{2A} receptors controlling neuroinflammation in rats subject to repeated restraint stress. *Front. Cell. Neurosci.* **2021**, *15*, 639322. [[CrossRef](#)] [[PubMed](#)]

94. Franco, R.; Cordoní, A.; Llinas Del Torrent, C.; Lillo, A.; Serrano-Marín, J.; Navarro, G.; Pardo, L. Structure and function of adenosine receptor heteromers. *Cell. Mol. Life Sci.* **2021**, *78*, 3957–3968. [[CrossRef](#)]
95. Shen, H.-Y.; Coelho, J.E.; Ohtsuka, N.; Canas, P.M.; Day, Y.-J.; Huang, Q.-Y.; Rebola, N.; Yu, L.; Boison, D.; Cunha, R.A.; et al. A critical role of the adenosine A_{2A} receptor in extrastriatal neurons in modulating psychomotor activity as revealed by opposite phenotypes of striatum and forebrain A_{2A} receptor knock-outs. *J. Neurosci.* **2008**, *28*, 2970–2975. [[CrossRef](#)] [[PubMed](#)]
96. Shen, H.-Y.; Canas, P.M.; Garcia-Sanz, P.; Lan, J.-Q.; Boison, D.; Moratalla, R.; Cunha, R.A.; Chen, J.-F. Adenosine A_{2A} receptors in striatal glutamatergic terminals and GABAergic neurons oppositely modulate psychostimulant action and DARPP-32 phosphorylation. *PLoS ONE* **2013**, *8*, e80902. [[CrossRef](#)]
97. Wei, C.J.; Augusto, E.; Gomes, C.A.; Singer, P.; Wang, Y.; Boison, D.; Cunha, R.A.; Yee, B.K.; Chen, J.-F. Regulation of fear responses by striatal and extrastriatal adenosine A_{2A} receptors in forebrain. *Biol. Psychiatry* **2014**, *75*, 855–863. [[CrossRef](#)]
98. Chen, J.-F.; Choi, D.-S.; Cunha, R.A. Striatopallidal adenosine A_{2A} receptor modulation of goal-directed behavior: Homeostatic control with cognitive flexibility. *Neuropharmacology* **2023**, *226*, 109421. [[CrossRef](#)]
99. Moreira-de-Sá, A.; Lourenço, V.S.; Canas, P.M.; Cunha, R.A. Adenosine A_{2A} receptors as biomarkers of brain diseases. *Front. Neurosci.* **2021**, *15*, 702581. [[CrossRef](#)]

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