Adenosine $\text{A}_2\text{A}$ receptors facilitate synaptic NMDA currents in CA1 pyramidal neurons

**Correspondence**  Ana Maria Sebastião, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Avenida Professor Egas Moniz, Lisbon 1649-028, Portugal. E-mail: anaseb@medicina.ulisboa.pt

**Received**  24 January 2018; **Revised**  18 July 2018; **Accepted**  21 August 2018

Francisco M Mouro$^{1,2,*}$, Diogo M Rombo$^{1,2,*}$, Raquel B Dias$^{1,2}$, Joaquim A Ribeiro$^{1,2}$ and Ana M Sebastião$^{1,2}$

$^1$Instituto de Farmacologia e Neurocéncias, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, and $^2$Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

*Co-first authors.

**BACKGROUND AND PURPOSE**

NMDA receptors play a key role in both synaptic plasticity and neurodegeneration. Adenosine is an endogenous neuromodulator and through membrane receptors of the $\text{A}_2\text{A}$ subtype can influence both synaptic plasticity and neuronal death. The present work was designed to evaluate the influence of adenosine $\text{A}_2\text{A}$ receptors upon NMDA receptor activity in CA1 hippocampal neurons. We discriminated between modulation of synaptic versus extrasynaptic receptors, since extrasynaptic NMDA receptors are mostly associated with neurodegeneration while synaptic NMDA receptors are linked to plasticity phenomena.

**EXPERIMENTAL APPROACH**

Whole-cell patch-clamp recordings were obtained to evaluate NMDA receptor actions on CA1 pyramidal neurons of young adult (5–10 weeks) male Wistar rat hippocampus.

**KEY RESULTS**

Activation of $\text{A}_2\text{A}$ receptors with CGS 21680 (30 nM) consistently facilitated chemically-evoked NMDA receptor-currents (NMDA-PSCs) and afferent-evoked NMDA-currents (NMDA-EPSCs), an action prevented by an $\text{A}_2\text{A}$ receptor antagonist (SCH58261, 100 nM) and a PKA inhibitor, H-89 (1 μM). These actions did not reflect facilitation in glutamate release since there was no change in NMDA-EPSCs paired pulse ratio. $\text{A}_2\text{A}$ receptor actions were lost in the presence of an open-channel NMDA receptor blocker, MK-801 (10 μM), but persisted in the presence of memantine, at a concentration (10 μM) known to preferentially block extrasynaptic NMDA receptors.

**CONCLUSION AND IMPLICATIONS**

These results show that $\text{A}_2\text{A}$ receptors exert a positive postsynaptic modulatory effect over synaptic, but not extrasynaptic, NMDA receptors in CA1 neurons and, therefore, under non-pathological conditions may contribute to shift the dual role of NMDA receptors towards enhancement of synaptic plasticity.

**Abbreviations**

aCSF, artificial CSF; CaMKII, Ca$^{2+}$/calmodulin-dependent protein kinase II; CGS 21680, 2-[4-(2-p-carboxyethyl)phenylamino]-S'N'-ethylcarbozamido adenosine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DL-AP5, 2-amino-5-phosphonovaleric acid; LTD, long-term depression; memantine, 3,5-dimethyl-1-adamantanamine hydrochloride; PPR, paired pulse ratio; SCH 58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; TTX, tetrodoxin citrate

DOI:10.1111/bph.14497 © 2018 The Authors. British Journal of Pharmacology published by John Wiley & Sons Ltd on behalf of British Pharmacological Society.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
**Introduction**

**NMDA receptors** are membrane-bound ionotropic glutamate receptors characterized by voltage-dependent activity, high permeability to calcium ions and slow activation/deactivation kinetics (Doherty and Sladek, 2011). Calcium influx through NMDA receptors is crucial for synaptogenesis, experience-dependent synaptic remodelling and for long-lasting changes in synaptic efficiency such as LTP and long-term depression (LTD). Indeed, NMDA receptors are key components in the induction of several forms of synaptic plasticity (Hunt and Castillo, 2012). Following depolarization of the postsynaptic membrane and release of magnesium blockade, a postsynaptic rise in calcium, mediated by the opening of NMDA receptors, activates important kinases such as calmodulin-dependent kinase II (CaMKII), PKA and PKC (Lau and Zukin, 2007), with an impact on synaptic reinforcement through fast signalling **AMPA receptors** at the postsynaptic membrane (Malenka and Nicoll, 1999; Morris et al., 2003; Collingridge et al., 2004). On the other hand, NMDA receptors are the main mediators of excitotoxicity, whenever there is an excessive and neurotoxic increase in intracellular calcium (Hardingham and Bading, 2010). The dual role of the NMDA receptor is related to receptor location relative to the synapse (Hardingham and Bading, 2010). Calcium influx through synaptic NMDA receptors is relatively well tolerated by neuronal cells and important for neuronal survival and synaptic plasticity, while an influx through extrasynaptic NMDA receptors is linked to neurotoxicity (Hardingham et al., 2002). Regulatory mechanisms that control NMDA receptor activity at specific membrane locations are, therefore, of particular importance and its knowledge relevant for strategies aiming to favour the balance towards neuronal survival and shape synaptic functioning.

**Adenosine** is an ubiquitous neuromodulator influencing the action and activity of several neurotransmitter receptors (Sebastião and Ribeiro, 2015). Adenosine A₁ receptors are responsible for the adenosine-mediated inhibitory effects, including inhibition of neurotransmitter release and changes in postsynaptic membrane conductance (Dunwiddle and Masino, 2001), as well as inhibition of NMDA receptor-mediated currents at hippocampal neurons (De Mendonça et al., 1995). The other subtype of high-affinity adenosine receptors, the adenosine A₂A receptors, is coupled to Gs proteins, classically leading to stimulation of adenylate cyclase, a rise in intracellular cAMP levels and PKA activation. A₂A receptors are mostly expressed in the basal ganglia but are also expressed in lower levels at the hippocampus where they exert important neuromodulatory actions such as the control of other modulators (Sebastião and Ribeiro, 2015) and regulate synaptic plasticity (Dias et al., 2013a). Indeed, adenosine A₂A receptors at the CA1 area of the hippocampus enhance LTP (Costenla et al., 2011; Dias et al., 2012), facilitate the actions of the brain-derived neurotrophic factor (BDNF) at hippocampal synapses (Diogènes et al., 2004; Fernandes et al., 2008) with consequences for synaptic plasticity (Fontinha et al., 2008), and enhance AMPA-evoked currents recorded from CA1 pyramidal neurons (Dias et al., 2012) with an impact on ischaemia-induced plasticity phenomena (Dias et al., 2013b). Also, the degree of activation of A₂A receptors affects hippocampal-dependent learning (Fontinha et al., 2009; Batalha et al., 2013), excitotoxicity and neuronal death (Jones et al., 1998; Cunha, 2016; Ribeiro et al., 2016).

The activity of A₂A receptors regulates the function of NMDA receptors in the striatum (Wirkner et al., 2004) and plays a permissive role in the positive interaction between metabotropic glutamate receptors 5 (mGlu₅ receptors) and NMDA receptors in the hippocampus (Tebano et al., 2005; Sarantis et al., 2015; Kouvaros and Papatheodoropoulos, 2016; Krania et al., 2018), an action recently shown to be involved in synaptic plasticity deregulation under conditions of presynaptic A₂A receptor overexpression (Temido-Ferreira et al., 2018). A₂A receptors’ activity is also required to gate a form of LTP mediated by postsynaptic NMDA receptors at mossy fibre hippocampal synapses (Rebola et al., 2008). However, despite the dual role of NMDA receptors, as well as of A₂A receptors upon LTP and excitotoxicity, no information so far exists on the direct influence of A₂A receptors on NMDA receptor-mediated currents. This issue is particularly important and needs to be investigated in the CA1 area of the hippocampus, given the major role of NMDA receptors in CA1 LTP and the vulnerability of this hippocampal area to excitotoxicity phenomena.

We herein evaluated whether activation of adenosine A₂A receptors could affect NMDA receptor-mediated currents in CA1 pyramidal cells. Given the differences between synaptic and extrasynaptic NMDA receptor-mediated activity upon neuronal function (Hardingham and Bading, 2010), we focused on distinguishing if any action produced by A₂A receptors detected mostly resulted from modulation of synaptic or extrasynaptic NMDA receptor-mediated currents.

**Methods**

**Hippocampal slice preparation and patch-clamp procedures**

Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny et al., 2010). All experiments were carried out on acute hippocampal slices obtained from 5- to 10-week-old male Wistar rats (Harlan Iberica, Spain). All experiments followed the European Community Guidelines (Directive 2010/63/EU) and the Portuguese Law (DL 113/2013) for Animal Care for Research Purposes, and were approved by the “Instituto de Medicina Molecular” Internal Committee and the Portuguese Animal Ethics Committee - Direcção Geral de Veterinária.

The animals were killed by decapitation under deep isoflurane anaesthesia. The brain was quickly removed, hemisected, and both hippocampi used to obtain transverse slices (300-μm-thick) cut on a Vibratome (VT 1000S; Leica, Nussloch, Germany) in ice-cold dissecting solution containing (in mM): sucrose 110; KCl 2.5; CaCl₂ 0.5; MgCl₂ 7; NaHCO₃ 25; NaH₂PO₄ 1.25; glucose 7, bubbled with 95% O₂ and 5% CO₂, pH 7.4. Slices were first incubated for 30 min at 35°C in artificial CSF (aCSF) containing (mM): NaCl 124; KCl 3; NaH₂PO₄ 1.25; NaHCO₃ 26; MgSO₄ 1; CaCl₂ 2 and glucose 10, pH 7.4, gassed with 95% O₂ and 5% CO₂.
after recovery for at least 1 h at room temperature, individual slices were set up for electrophysiological recordings. Throughout the recording period, the aCSF was supplemented with 5 μM glycine and no Mg²⁺ was added.

Individual slices were fixed with a grid in a recording chamber and continuously superfused by a gravitational superfusion system at 2–3 mL·min⁻¹ with aCSF (with glycine and no Mg²⁺) at room temperature. Unless stated otherwise, drugs were added to the superfusion solution. Zero minutes in the figures correspond to the time when the first test solution was changed and indicate time at which the transfer was initiated. Around 2–3 min elapsed before the new solution reached the recording chamber; it took several minutes before the final concentration of the test drug was achieved in the recording chamber (dead volume of the recording chamber plus perfusion tubing: 10 mL).

Patch pipettes (4–9 MΩ) were pulled from borosilicate glass capillaries (1.5 mm outer diameter, 0.86 inner diameter, Harvard Apparatus), with a PC-10 Puller (Narishige Group) and filled with an intracellular solution containing (mM): K-glucuronate 125; KCl 11; CaCl₂ 0.1; MgCl₂ 2; EGTA 1; HEPES 10; MgATP 2; NaGTP 0.3 and phosphocreatine 10, pH 7.3, adjusted with KOH (1 M), 280-290 mOsm.

Electrode positioning and cell selection were performed under visual guidance using a Carl Zeiss Axioskop 2FS upright microscope (Jena, Germany) equipped with a differential interference contrast-infrared (DIC-IR) CCD video camera (VX44, Till Photonics, Gräfelfing, Germany).

Recordings were performed in voltage-clamp mode (VH = −60 mV) with an EPC-7 amplifier (List Biologic, Campbell, CA) or Axopatch 200B amplifier (Axon Instruments, CA, USA). The junction potential was not compensated for, and offset potentials were nullified before giga-seal formation. Small voltage steps (5 mV, 50 ms) were delivered throughout the experiment to monitor the access resistance; the holding current was also constantly monitored, and when any of these parameters varied by more than 20%, the experiment was rejected. The current signal was low-pass filtered using a 3 and 10 kHz three-pole Bessel filter, digitized at 10 kHz using a Digidata 1322A board and registered by the Clampex software version 10.2 (Molecular Devices, Sunnyvale, CA, USA). Data analysis was performed with Clampfit 10 (included in pCLAMP 10).

NMDA receptor-mediated postsynaptic currents (NMDA-PSCs) were evoked through an NMDA (150 μM)-filled micropipette coupled to a pressure application system (PicoPump PV820; World Precision Instruments) positioned close to the cell soma of the recording cell and injecting single pulses of 10 ms, 6–10 μL, applied every 2 min. The concentration of NMDA in the micropipette, the frequency of stimulation, injection duration and pressure were chosen within a range used by others (Khosravani et al., 2008; Papouin et al., 2012; Laprell et al., 2015) to obtain stable NMDA-PSCs recordings under control conditions. The pressure of injection was adjusted from experiment to experiment, as needed to obtain a stable recording, and once defined, it was not changed up to the end of the experiment. Individual current recordings were used for analysis, and their amplitude is depicted in the figure time course panels. Current tracings in the figures are averages of five consecutive individual recordings, thus corresponding to averaged recordings for 10 min before test drug application and for 10 min during peak drug effect. In NMDA-PSC recording experiments, tetrodotoxin citrate (TTX) was added to aCSF to prevent activity-dependent neurotransmitter release and minimize presynaptic interference of the recordings.

Afferent-evoked EPSCs mediated by NMDA receptors (NMDA-EPSCs) were obtained by stimulating the Schaffer Collateral afferents with 0.2 ms rectangular pulses delivered once every 15 s through a concentric electrode (Harvard) placed in the stratum radiatum. Averages of eight consecutive individual recordings (2 min) were used for analysis. The aCSF was supplemented with CNQX (10 μM), to block AMPA and kainate glutamate receptors and gabazine (1 μM), to block GABA_A receptor activity. In paired-pulse experiments, recordings were obtained with the same condition as single-pulse recordings but two consecutive NMDA-EPSCs were evoked 100 ms apart.

In all experiments, whenever assessing the influence of a drug on NMDA receptor-mediated currents, the test drug was superfused in the aCSF only after at least 10 min of stable (non-continuous change in the same direction) recordings of current amplitude. The averaged amplitude values during the 10 min before test drug application were normalized to 100% (baseline amplitude), to allow comparison of the magnitude of drug effects in different experiments; the effect of the test drug was quantified as percentage change from that of baseline.

**Drugs**

CGS 21680 (a selective A2A receptor agonist), SCH 58261 (a selective and competitive A2A receptor antagonist), H-89 dihydrochloride hydrate (a selective inhibitor of cAMP-dependent protein kinase) and (+)-MK-801 hydrogen maleate (dizocilpine; a non-competitive NMDA antagonist acting at the ion channel site) were obtained from Sigma-Aldrich (MO, USA). NMDA, DL-AP5 (a selective NMDA receptor antagonist) and gabazine (SR-95531; a selective and competitive GABA_A receptor antagonist) were obtained from Ascent Scientific (Bristol, UK), TTX (sodium-channel blocker) and CNQX, an antagonist of AMPA/Kainate receptors, were obtained from Tocris Bioscience (Bristol, UK).

Memantine was obtained from Abcam Biochemicals (Cambridge, UK). Isoflurane was obtained from Abbot Laboratories (Barcelona, Spain) and 1,2-propylenylcyle from Merck (NJ, USA). CGS 21680 (5 mM) and CNQX (100 mM) were prepared as a stock solution in DMSO. TTX (1 mM) was prepared in water, as well as NMDA (100 μM), DL-AP5 (50 mM), gabazine (5 mM), MK-801 (10 mM) and memantine (100 mM). Stock solutions were aliquoted and stored at −20°C until use. Dilutions of these stock solutions to the final concentration (in aCSF) were made freshly before each experiment.

**Statistical analysis**

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). Data presented were tested for normality (Shapiro–Wilk test), and when passed, parametric analyses were performed. Statistical significance was evaluated by Students two-tailed paired t-test (parametric) or Wilcoxon matched-pairs signed rank test (nonparametric),
A2A receptors facilitate synaptic NMDA currents

Table 1
Comparison between the effect of the A2A receptor agonist, CGS 21680 (30 nM), on NMDA currents at CA1 pyramidal neurons

| Condition                  | % of change |
|----------------------------|-------------|
| NMDA-PSCs                  | 133 ± 13 (n = 6; N = 6) |
| NMDA-PSCs (with MEM)       | 152 ± 15 (n = 7; N = 4) |
| NMDA-EPSCs (no MEM)        | 135 ± 5.2 (n = 11; N = 6) |
| NMDA-EPSCs (with MEM)      | 139 ± 9.7 (n = 8; N = 4) |

The values represent the action of CGS 21680 (30 nM) on NMDA currents when compared to baseline level (10 min before drug perfusion, P < 0.05. Students paired t-test). The number of experiments (n) and the number of animals used (N) for each condition are shown in brackets. The magnitude of the effect did not differ among different conditions (multiple comparison one-way ANOVA was not statistically significant, P = 0.82). MEM, memantine.

while comparing data from periods before and after drug perfusion. One-way ANOVA, with the Greenhouse–Geisser correction, followed by Tukey’s multiple comparison post test (parametric) or Friedman test followed by a Dunn’s multiple comparisons test (nonparametric) was used for multiple comparisons. All statistical analyses were performed in PRISM GraphPad 6.0 (La Jolla, CA, USA). Statistical significance was assumed when the P value was below 0.05. All data are presented as mean ± SEM of n experiments, where n corresponds to the number of recorded cells from n hippocampal slices (only one cell for each slice was used in each experiment). The number of animals used for each set of experiments (N) is also indicated in the figure legends and Table 1.

Nomenclature of targets and ligands
Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently recorded in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017a,b,c).

Results
Activation of adenosine A2A receptors facilitates NMDA-PSCs in CA1 pyramidal neurons
To study the actions of A2A receptor activation on NMDA receptor function, we recorded CA1 pyramidal cells in whole-cell voltage-clamp mode (Vh = −60 mV) in hippocampal slices. NMDA receptor currents were evoked by pressure applying NMDA (150 μM, 10 ms) close to the soma of the recorded cell every 2 min (NMDA-PSCs). After a stable baseline, the A2A agonist, CGS 21680, was applied at a concentration (30 nM) selective for A2A receptors (Jarvis et al., 1989), resulting in an increase in NMDA-PSCs amplitude that was already visible 2–3 min after starting the changeover of the solution and reached a maximum after 40 min of perfusion (to 133 ± 13% of baseline level; Figure 1A,D, Table 1). This is in accordance with the slow kinetics of CGS 21680 binding to its receptor (Hothersall et al., 2017) and the time for activation of GPCR and kinase-dependent signalling pathways (Lohse et al., 2008). Following the maximum effect, the amplitude of NMDA-PSCs started to decrease while continuously perfusing CGS 21680 and returned to near pre-control levels (to 115 ± 8.4% of baseline) within 20–30 min after CGS 21680 washout. The effects of the A2A agonist upon NMDA-PSCs recorded from slices 5- to 7- or 8- to 10-week-old rats did not differ appreciably. Both rise time (measured from 10 to 90% of peak value) and decay time (decay to 50% of peak value) of NMDA-PSCs were not changed during perfusion of CGS 21680 (Figure 1B, Table 2) indicating no change in NMDA-PSCs kinetics (rise time change from baseline: 7.3 ± 9.4%; decay time change from baseline: 1.2 ± 9.4%).

To assess if the facilitatory action of CGS 21680 could be attributed to A2A receptor activation, we tested if this action could be prevented by the selective A2A antagonist SCH58261 (100 nM; Zocchi et al., 1996; Rombo et al., 2015). For this purpose, SCH58261 was added to the perfusion for at least 30 min before adding the agonist to allow equilibrium with the antagonist before assessing the effect of the agonist. The presence of SCH58261 fully prevented the facilitatory action of CGS 21680 (% change by the agonist: to 105 ± 4.7% of baseline; Figure 1C,D), strongly suggesting a selective A2A receptor-mediated effect on NMDA-PSCs. By itself, SCH58261 (100 nM) did not affect NMDA-PSCs amplitude (% change by the antagonist: to 104 ± 13% of baseline; Figure 1C,D) suggesting the lack of endogenous action of adenosine on NMDA receptor-mediated currents, under the experimental conditions used.

Given that A2A receptor activation with CGS 21680 can also facilitate AMPA receptor-mediated currents, enhancing surface expression of Glu1 receptor subunits (Dias et al., 2012), we excluded the contribution of AMPA receptors for the recorded PSCs by perfusing the AMPA/kainate receptor antagonist, CNQX (10 μM), with no effect on NMDA-PSC (to 99 ± 4.5% of baseline; Figure 1E). Consistent with a selective NMDA receptor-mediated current, the perfusion of the NMDA receptor antagonist, DL-AP5 (50 μM), caused a fast decrease in NMDA-PSCs amplitude to 19 ± 2.3% of baseline after 10 min of perfusion (Figure 1E).

Knowing that, in the young-adult brain, three quarters of NMDA receptors are extrasynaptically located (Hardingham and Bading, 2010), it is likely that extrasynaptic NMDA receptors greatly contribute to NMDA-PSCs evoked by pressure application of the drug over the neuronal cell body. Thus, we performed a set of experiments testing the actions of memantine, a drug that, at selected concentrations, preferentially blocks extrasynaptic NMDA receptors (Xia et al., 2010). Compatible with an important contribution of extrasynaptic NMDARs NMDA receptors to NMDA-PSCs, perfusion of memantine (1 μM) significantly decreases NMDA-PSCs to 49 ± 3.2% of baseline (Figure 1F,G). No statistically significant change in NMDA-PSC kinetics was observed (Table 2). In the presence of memantine, CGS 21680 (30 nM) was still able to increase NMDA-PSCs (to 152 ± 15% of baseline values recorded before applying CGS 21680; Figure 1F inset, Figure 1G, Table 1), suggesting that the facilitatory action of A2A receptors upon NMDA receptor activity in CA1 pyramidal cells does not occur exclusively through extrasynaptic NMDA receptors.

British Journal of Pharmacology (2018) 175 4386–4397 4389
Figure 1

A2A receptor activation enhances chemical-evoked NMDA-PSCs. (A) Average normalized time course changes of the effect of 30 nM CGS 21680 (horizontal bar), a selective A2A agonist, on chemically-evoked NMDA-PSCs peak amplitude (n = 6, N = 6). Zero time in the abscissa corresponds to the time when the test solution started to be perfused. Data are presented as mean ± SEM. Top insets: tracings (average of five consecutive currents, 10 min) obtained from representative experiments before (1) and 40 min after (2) CGS 21680 superfusion. *P < 0.05, baseline versus CGS 21680 effect (Wilcoxon matched-pairs test). (B) Rise time (measured from 10 to 90% of peak value, ms) and decay time (decay to 50% of peak value, ms) values (left and right panels, respectively) for each individual experiment, with a line connecting values at baseline and after 40 min of CGS 21680 perfusion. Histogram depicts mean ± SEM for each set of values. ns, not significant (rise time, n = 6, P = 0.84 and decay time, n = 6, P = 0.63, Wilcoxon matched-pairs test). (C) Time course of averaged normalized changes in NMDA-PSC peak amplitude induced by superfusion of SCH58261 (100 nM) (a selective A2A antagonist) (n = 3, N = 3, left panel) and CGS 21680 (30 nM) while in the presence of SCH58261 (100 nM) (n = 4, N = 4, right panel). ns, not significant; left panel: baseline versus CGS 21680 (n = 3, P = 0.25); right panel: SCH 58261 versus SCH 58261 + CGS 21680 (n = 4, P = 0.13) (Wilcoxon matched-pairs test). (D) Summary plot showing NMDA-PSC peak amplitude (pA) of all individual cells shown in (A) and (C). (E) Averaged normalized time course changes of the effect of CNQX (10 μM; upper horizontal bar) and DL-AP5 (50 μM; bottom horizontal bar) on NMDA-PSC peak amplitude. (F) Average normalized time course changes of the effect of memantine (MEM; 1 μM, preferentially extrasynaptic NMDA receptor antagonist, upper horizontal bar) and CGS 21680 (30 nM, a selective A2A agonist, bottom horizontal bar), on chemically-evoked NMDA-PSCs peak amplitude (n = 7, N = 4); data presented as mean ± SEM; inset (obtained from the period limited by dashed line rectangle) highlights the effect of CGS 21680 normalized to baseline already in the presence of memantine (baseline recalculated from 40 to 50 min of time course). Right upper inset: sample traces (average of five consecutive currents, 10 min) taken from a representative experiment during baseline (1), memantine superfusion (2) and memantine + CGS 21680 superfusion (3). Right bottom panel: summary plot showing NMDA-PSC peak amplitude (pA) of all individual cells shown in left panel. (G) Baseline normalized NMDA-PSC amplitude values for each individual experiment, with a line connecting values from memantine perfusion and memantine + CGS 21680 perfusion. Histogram depicts mean ± SEM for each set of values. *P < 0.05; n = 7, N = 4, one-way ANOVA followed by Tukey’s multiple comparisons test.
**Table 2**

Comparison between the rise and decay times of NMDA receptor-mediated currents in different recordings and conditions

| Recordings                | Condition                  | Rise time (ms) | Statistics (vs. baseline) | Decay time (ms) | Statistics (vs. baseline) |
|---------------------------|----------------------------|----------------|--------------------------|-----------------|--------------------------|
| NMDA-PSC (Figure 1A)     | Baseline                  | 146 ± 36       | \( n = 6, P = 0.84 \)    | 591 ± 112       | \( n = 6, P = 0.63 \)    |
|                           | CGS 21680 (30 nM)          | 147 ± 31       |                          | 571 ± 104       |                          |
| NMDA-PSC (MEM) (Figure 1F)| Baseline                  | 188 ± 27       | \( n = 7, P = 0.94 \)    | 650 ± 124       | \( n = 7, P = 0.94 \)    |
|                           | MEM (1 μM)                | 190 ± 39       |                          | 653 ± 149       |                          |
| NMDA-EPSC (1stP) (Figure 2A)| Baseline             | 14 ± 2.8       | \( n = 11, P = 0.95 \)   | 32 ± 4.2        | \( n = 7, P = 0.81 \)    |
|                           | CGS 21680                 | 14 ± 2.7       |                          | 32 ± 4.3        |                          |
| NMDA-EPSC (2ndP) (Figure 2A)| Baseline            | 12 ± 1.7       | \( n = 11, P = 0.98 \)   | 33 ± 4.0        | \( n = 7, P = 0.84 \)    |
|                           | CGS 21680                 | 12 ± 1.6       |                          | 33 ± 3.8        |                          |
| NMDA-EPSC (MK-801) (Figure 3A) | Baseline              | 22 ± 1.9       | \( n = 10, P = 0.05 \)   | 37 ± 3.1        | \( n = 10, P = 0.71 \)   |
|                           | MK-801 (10 μM)            | 23 ± 2.9       |                          | 36 ± 3.0        |                          |
|                           | MK-801 + CGS 21680        | 19 ± 1.7       |                          | 35 ± 3.9        |                          |
| NMDA-EPSC (MEM) (Figure 3D)| Baseline              | 40 ± 4.2       | \( n = 8, P = 0.11 \)    | 76 ± 5.3        | \( n = 8, P = 0.13 \)    |
|                           | MEM (1 μM)                | 36 ± 4.7       |                          | 71 ± 3.8        |                          |
|                           | MEM + CGS 21680           | 31 ± 5.6       |                          | 72 ± 5.0        |                          |

No statistically significant changes were observed in any of the conditions evaluated. 1stP, first pulse; 2ndP, second pulse; MEM: memantine.

**Activation of adenosine \( A_{2A} \) receptors predominantly facilitates synaptic NMDA receptor activity**

The results presented above suggest that synaptic-located NMDA receptors are targeted by adenosine \( A_{2A} \) receptors. To confirm this, we evaluated whether CGS 21680 could enhance NMDA currents when the receptors predominantly contributing to the currents were indeed synaptic NMDA receptors. We thus recorded afferent-evoked EPSCs by electrically stimulating in *stratum radiatum* in the continuous presence of CNQX (10 μM) and gabazine (5 μM), to block AMPA/kainate and GABA\(_A\) receptors respectively. Under such conditions, the facilitatory effect of the \( A_{2A} \) agonist, CGS 21680 (30 nM), was maintained, enhancing NMDA-EPSCs amplitude to 135 ± 5.2% of baseline (Figure 2A,B, Table 1).

Again, the effect of the \( A_{2A} \) agonist upon NMDA-EPSCs amplitude to 135 ± 5.2% of baseline (Figure 2A,B, Table 1).

The CGS 21680 effect was not accompanied by changes in rise and decay times of both first and second pulses (rise time change from baseline: 2.2 ± 3.2% for first pulse and 2.3 ± 6.6% for second pulse; decay time change from baseline: 0.1 ± 4.8% for first pulse and 0.7 ± 3.9% for second pulse; Figure 2C, Table 2).

As expected, the NMDA-EPSCs were fully blocked by the perfusion of the NMDA antagonist DL-AP5 (50 μM) (currents decreased to 7.9 ± 1.2% of baseline; Figure 2D), confirming the NMDA-mediated nature of the evoked responses.

Altogether, the above experiments suggest that synaptic NMDA receptors, rather than extrasynaptic ones, are targeted by \( A_{2A} \) receptors. To further assess this possibility, the next series of experiments were designed to better discriminate between \( A_{2A} \) receptor modulation over synaptic or extrasynaptic NMDA receptors.

We first evaluated the possibility that peri and extrasynaptic NMDA receptors could be contributing to the recorded EPSCs, since they can be recruited either by glutamate spillover into extrasynaptic compartments or during intense synaptic activity. Thus, we recorded NMDA-EPSCs (0.067 Hz, paired-pulse) and perfused an open-channel NMDA receptor blocker, MK-801 (10 μM), to use-dependently inhibit synthetically evoked NMDA-EPSCs and isolate extrasynaptically recruited NMDA receptors.

In fact, MK-801 perfusion decreased NMDA-EPSCs to 31 ± 1.9% of baseline (Figure 3A,B), without altering NMDA receptor current kinetics (Table 2), revealing a strong component of synaptic NMDA receptor activity on electrical-evoked stimulation conditions. We then perfused CGS 21680 (30 nM) in the presence of MK-801, aiming to activate \( A_{2A} \) receptors in conditions in which extrasynaptic NMDA receptors are the ones mostly recruited. Under these conditions CGS 2160 caused no statistically significant changes on NMDA-EPSCs amplitude (to 111 ± 12.6% of baseline values recorded before applying CGS 2160; Figure 3A,B) or current kinetics.
Table 2), indicating the lack of A2A receptor action upon extrasynaptic NMDA receptors.

We next performed a set of experiments testing the actions of memantine on afferent-evoked NMDA-EPSCs. Perfusion of memantine (1 μM) caused no significant change in NMDA-EPSCs amplitude (to 96 ± 4.8% of baseline; Figure 3C,D) or kinetics (Table 2). Consistent with A2A receptor action being preferentially upon synaptic NMDA receptors, perfusion of CGS 21680 in the presence of memantine lead to a facilitation of NMDA-EPSCs to 139 ± 9.7% of baseline values recorded before perfusion of CGS21680 (Figure 3C,D), with no change in current kinetics (Table 2), similar to what was observed in the absence of memantine (Figure 2A, Table 1).

Finally, we tested for the dependency of A2A receptors actions on classical signalling pathway involving cAMP/PKA activation (Fredholm et al., 2011). To test this, we first perfused a selective inhibitor of cAMP-dependent protein kinase, H-89, at a concentration (1 μM) that causes marked inhibition of PKA (Bain et al., 2007) without inhibiting other kinases that could possibly be involved in the synaptic actions of A2A receptors, such as ERK or MAPK, PKCα or CAMK (Bain et al., 2007). H-89, per se, had no effect on NMDA-EPSCs (to 97 ± 2.8% of baseline before H-89; Figure 3E,F) but completely prevented the action of CGS 21680 on NMDA-EPSCs (to 108 ± 3.8% of baseline before adding CGS 21680; Figure 3E,F), a finding compatible with the involvement of canonical pathway for A2A receptors, the cAMP/PKA signalling cascade, in the modulating effect of A2A receptors on NMDA receptors.

Altogether, our results strongly indicate a preferential action of A2A receptors on synaptic and not extrasynaptic NMDA receptors via cAMP/PKA signalling.
Figure 3

Synaptic, but not extrasynaptic, NMDA-EPSCs are affected by adenosine A2A receptor activation through cAMP/PKA signalling. (A) Average normalized time course changes of the effect of MK-801 (10 μM, an open-channel NMDA receptor blocker, upper horizontal bar) and CGS 21680 (30 nM, a selective A2A receptor agonist, bottom horizontal bar), on electrically-evoked NMDA-EPSCs peak amplitude (n = 10, N = 5). Zero time in the abscissa corresponds to the time when the first test solution started to be perfused. Data are presented as mean ± SEM; inset (obtained from the period limited by dashed line rectangle) highlights the effect of CGS 21680 normalized to baseline already in the presence of MK-801 (baseline recalculated from 35 to 45 min of time course). Right upper inset: sample traces (average of 8 consecutive currents, 2 min) taken from a representative experiment during baseline (1), MK-801 superfusion (2) and MK-801 + CGS 21680 superfusion (3); the stimulus artefact for each trace was removed for better visualization. Right bottom panel: summary plot showing NMDA-EPSC peak amplitude (pA) of all individual cells shown in left panel. (B) Baseline-normalized NMDA-EPSC amplitude values for each individual experiment, with a line connecting values during MK-801 perfusion and MK-801 + CGS 21680 perfusion. Histogram depicts mean ± SEM for each set of values. *P < 0.05; ns, not significant, n = 10, N = 5, one-way ANOVA followed by Tukey’s multiple comparisons test. (C) Average normalized time course changes for the effect of memantine (1 μM, preferentially extrasynaptic NMDA receptor antagonist, upper horizontal bar) and CGS 21680 (30 nM, a selective A2A receptor agonist, bottom horizontal bar), on electrically-evoked NMDA-EPSCs peak amplitude (n = 8, N = 4); data presented as mean ± SEM; inset (obtained from the period limited by dashed line rectangle) highlights the effect of CGS 21680 normalized to baseline already in the presence of Memantine (baseline recalculated from 35 to 45 min of time course). Right upper inset: sample traces (average of eight consecutive currents, 2 min) taken from a representative experiment during baseline (1), membrane superfusion (2) and memantine + CGS 21680 superfusion (3); the stimulus artefact for each trace was removed for better visualization. Right bottom panel: summary plot showing NMDA-EPSC peak amplitude (pA) of all individual cells shown in left panel. (D) Baseline-normalized NMDA-EPSC amplitude values for each individual experiment, with a line connecting values during memantine perfusion and memantine + CGS 21680 perfusion. Histogram depicts mean ± SEM for each set of values. ns, not significant; *P < 0.05; n = 8, N = 4, one-way ANOVA followed by Tukey’s multiple comparisons test. (E) Average normalized time course changes of the effect of H-89 (1 μM, a selective inhibitor of cAMP-dependent protein kinase, upper horizontal bar) and CGS 21680 (30 nM, a selective A2A agonist, bottom horizontal bar), on electrically-evoked NMDA-EPSCs peak amplitude (n = 5, N = 3); data presented as mean ± SEM. Right upper inset: sample traces (average of eight consecutive currents, 2 min) taken from a representative experiment during baseline (1), H-89 superfusion (2) and H-89 + CGS 21680 superfusion (3); the stimulus artefact for each trace was removed for better visualization. Right bottom panel: summary plot showing NMDA-EPSC peak amplitude (pA) of all individual cells shown in left panel. (F) Baseline-normalized NMDA-EPSC amplitude values for each individual experiment, with a line connecting values during H-89 perfusion and H-89 + CGS 21680 perfusion. Histogram depicts mean ± SEM for each set of values. ns, not significant; n = 5, N = 5, Kruskal–Wallis test followed by Dunn’s multiple comparisons test.
Discussion

The main finding in the present work is that adenosine A$_{2A}$ receptors facilitate postsynaptic NMDA currents in CA1 pyramidal neurons and that this facilitation preferentially occurs at the synaptic rather than extrasynaptic level.

A$_{2A}$ receptors at the hippocampus are scarcely distributed but are known to affect hippocampal synaptic transmission at different levels. A predominant role of hippocampal A$_{2A}$ receptors is to facilitate the action of other neurotransmitters, such as BDNF (Diógenes et al., 2004), or receptors for neurotransmitters, such as glutamate AMPA receptors (Dias et al., 2012) and mGlu$_5$ receptors (Tebano et al., 2005). Presynaptically, A$_{2A}$ receptors are also known to increase glutamate release, although in young rats this action is small and mostly results from a blockade of A$_1$ receptor-mediated inhibitory effect (Lopes et al., 2002). In addition, A$_{2A}$ receptors at the hippocampus also enhance GABA release (Cunha and Ribeiro, 2000), an action that is synapse-specific resulting in a disinhibition of glutamatergic neurons (Rombo et al., 2015). All these A$_{2A}$ receptor-mediated actions may impact synaptic plasticity (Dias et al., 2013a; Sebastião and Ribeiro, 2015), as demonstrated by A$_{2A}$ receptor facilitation of LTP (Costenla et al., 1999; Dias et al., 2012) and post-ischaemia plasticity phenomena (Dias et al., 2013b). The influence of A$_{2A}$ receptors on hippocampal NMDA receptors has been much less explored. There is, however, evidence on dentate gyrus-CA3 synapses that A$_{2A}$ receptor activation unmasks a new form of NMDA receptor-dependent LTP that most likely occurs at the postsynaptic level (Rebola et al., 2008). However, evidence for a direct postsynaptic influence of A$_{2A}$ receptors upon NMDA receptors was until now lacking. The data obtained in the present study have revealed this missing link, clearly showing that NMDA receptors are targets for A$_{2A}$ receptors likely through the canonical cAMP/PKA signalling pathway, and importantly, we showed that this action is evident in young animals and occurs predominantly at synaptic rather than extrasynaptic receptors. Furthermore, this action was clearly detected in the CA1 hippocampal area where synaptic plasticity mostly depends upon NMDA receptors.

A$_{2A}$ receptors are known to affect memory tasks dependent on intact hippocampal activity, an action that seems to depend on the age and physiological state of the animal. Thus, while in young animals, A$_{2A}$ receptor blockade disturbs hippocampal-dependent conditional learning as well as prevents the concomitant increase in synaptic potentials at the hippocampus (Fontinha et al., 2009), supporting a facilitatory action of A$_{2A}$ receptor synaptic plasticity and memory acquisition, in aged animals, where spurious LTP occurs at Schaffer CA1 synapses (Diógenes et al., 2007), A$_{2A}$ receptor blockade restores plasticity towards normal values (Costenla et al., 2011). The same also occurs in chronically stressed animals where A$_{2A}$ receptor blockade has been shown to counteract exacerbated plasticity and to facilitate learning (Batalha et al., 2013), thus suggesting a negative influence of A$_{2A}$ receptors upon hippocampal-dependent tasks. This apparent contradiction on the influence of A$_{2A}$ receptors in learning in young versus aged and/or diseased animals may result from the different roles of A$_{2A}$ receptors in healthy young animals, aged animals or under disease conditions (Sebastião and Ribeiro, 2009). The same is also known to occur with NMDA receptors. Indeed, it is widely accepted that synaptic NMDA receptor activation is necessary for several forms of memory encoding and retrieval. Thus, our results showing that A$_{2A}$ receptors can facilitate synaptic NMDA receptors in young animals help to explain the negative effects of A$_{2A}$ receptor antagonists on certain learning paradigms in healthy young animals.

In contrast, neurodegenerative conditions are known to increase activation of NMDA receptors and calcium-permeable AMPA receptors, leading to hippocampal synaptic plasticity impairment (Diógenes et al., 2012; Ferreira et al., 2017; Tanqueiro et al., 2018). There is plenty of evidence that under pathological conditions, A$_{2A}$ receptor signalling is exacerbated, which contributes to pathogenesis and leads to early cognitive and synaptic dysfunction as well as neurodegeneration (Canas et al., 2009; Cognato et al., 2010; Batalha et al., 2013; Kadowaki-Horita et al., 2013; Kaster et al., 2015; Li et al., 2015a,b; Hu et al., 2016; Laurent et al., 2016; Viana da Silva et al., 2016). Under such pathological conditions, A$_{2A}$ receptor blockade restores learning and/or memory (Canas et al., 2009; Cognato et al., 2010; Batalha et al., 2013; Kadowaki-Horita et al., 2013; Kaster et al., 2015; Li et al., 2015b; Viana da Silva et al., 2016). A$_{2A}$ receptor-mediated dysfunctions may even precede NMDA receptor dysfunction (Viana da Silva et al., 2016). In most of the above-mentioned studies, the dysfunctional A$_{2A}$ receptor activity predominantly occurs at the presynaptic level, enhancing glutamate release, therefore, being prone to indirectly facilitate NMDA receptor activation, which under pathological conditions may provide a positive feedback loop towards neurodegeneration. Interestingly, in healthy aged animals, enhanced synaptic plasticity and NMDA receptor activation seem to compensate cognitive decline (Pinho et al., 2017). One may thus think that while in disease conditions, A$_{2A}$ receptors exacerbate excitotoxicity and, under non-diseased conditions, a facilitatory action of A$_{2A}$ receptors on several steps of excitatory synaptic transmission may compensate some loss of function in the aged. Importantly, since synaptic and extrasynaptic NMDA receptors have different roles in plasticity and in neurodegeneration, with the extrasynaptic NMDA receptors being more prone to gate neurodegeneration and synaptic NMDA receptors mostly involved in synaptic plasticity (Hardingham and Bading, 2010), a drug that could selectively facilitate synaptic NMDA receptors without affecting extrasynaptic ones could be a candidate to selectively promote plasticity with minimal consequences on degeneration. Selective A$_{2A}$ receptor agonists may prove to usefully fulfil this task.

In conclusion, our results showing that A$_{2A}$ receptors predominantly affect synaptic NMDA receptors thus suggest that, at least in young animals, A$_{2A}$ receptor activation may contribute to a shift in the dual role of NMDA receptors towards enhancement of synaptic plasticity. Further studies in healthy aged animals are desirable to clarify weather this selective influence of A$_{2A}$ receptors on synaptic NMDA receptors, rather than extrasynaptic receptors, may prove beneficial to enhance cognition without triggering neurodegeneration.
Acknowledgements

Work supported by LISBOA-01-0145-FEDER-007391, project co-funded by FEDER through POR Lisboa 2020 (Programa Operacional Regional de Lisboa) from PORTUGAL 2020 and Fundação para a Ciência e Tecnologia, (FCT), by an FCT project (PTDC/DTP-FTO/3346/2014), and by a Twinning action (SynaNet) from the EU H2020 programme (project number: 692340). F.M.M. was in receipt of SFRH/BD/89582/2012 FTC fellowship, and D.M.R. is in receipt of a fellowship from Instituto de Medicina Molecular (IMM/BI/38-2017).

Author contributions

F.M.M. and D.M.R. conducted the experiments, data acquisition and analysis. R.B.D. and D.M.R. introduced F.M.M. to technical procedures, data acquisition and analysis. All authors were involved in the design of the experiments. A.M.S. designed the project. F.M.M., D.M.R. and A.M.S. wrote the manuscript. All authors contributed to the final version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

References

Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA et al. (2017a). The Concise Guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. Br J Pharmacol 174: S17–S129.

Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Facenda E et al. (2017b). The Concise Guide to PHARMACOLOGY 2017/18: Enzymes. Br J Pharmacol 174: S272–S359.

Alexander SPH, Peters JA, Kelly E, Marrion NV, Facenda E, Harding SD et al. (2017c). The Concise Guide to PHARMACOLOGY 2017/18: Ligand-gated ion channels. Br J Pharmacol 174: S130–S159.

Batalha VL, Pego JM, Fontinha BM, Costenla AR, Valadas JS, Baqi Y et al. (2013). Adenosine A2A receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. Mol Psychiatry 18: 320–331.

Bain J, Plater L, Elliott M, Shipiro N, Hastie CJ, McIauchlan H et al. (2007). The selectivity of protein kinase inhibitors: a further update. Biochem J 408: 297–315.

Canas PM, Forciuncula LO, Cunha GM, Silva CG, Machado NJ, Oliveira JM et al. (2009). Adenosine A2A receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. J Neurosci 29: 14741–14751.

Cognato GP, Agostinho PM, Hockemeyer J, Müller CE, Souza DO, Cunha RA (2010). Caffeine and an adenosine A2A receptor antagonist prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. J Neurochem 112: 453–462.

Collingridge GL, Isaac JTR, Wang YT (2004). Receptor trafficking and synaptic plasticity. Nat Rev Neurosci 5: 952–962.

Costenla AR, De Mendonça A, Ribeiro JA (1999). Adenosine modulates synaptic plasticity in hippocampal slices from aged rats. Brain Res 851: 228–234.

Costenla AR, Diógenes MJ, Canas PM, Rodrigues Rj, Nogueira C, Maroco J et al. (2011). Enhanced role of adenosine A2A receptors in the modulation of LTP in the rat hippocampus upon aging. Eur J Neurosci 34: 12–21.

Cunha RA, Ribeiro JA (2000). Purinergic modulation of [3H] GABA release from rat hippocampal nerve terminals. Neuropharmacology 39: 1156–1167.

Cunha RA (2016). How does adenosine control neuronal dysfunction and neurodegeneration? J Neurochem 139: 1019–1055.

Curtis MJ, Bond RA, Spina D, Aihuwallia A, Alexander SPA, Giembycz MA et al. (2015). Experimental design and analysis and their reporting: new guidance for publication in BJP. Br J Pharmacol 172: 3461–3471.

De Mendonça A, Sebastião AM, Ribeiro JA (1995). Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurons by adenosine A1 receptor activation. Neuroreport 6: 1097–1100.

Dias RB, Ribeiro JA, Sebastião AM (2012). Enhancement of AMPA currents and GluR1 membrane expression through PKA-coupled adenosine A2A receptors. Hippocampus 22: 276–291.

Dias RB, Rombo DM, Ribeiro JA, Henley JM, Sebastião AM (2013a). Adenosine: setting the stage for plasticity. Trends Neurosci 36: 248–257.

Dias RB, Rombo DM, Ribeiro JA, Sebastião AM (2013b). Ischemia-induced synaptic plasticity drives sustained expression of calcium-permeable AMPA receptors in the hippocampus. Neuropharmacology 65: 114–122.

Diógenes MJ, Fernandes CC, Sebastião AM, Ribeiro JA (2004). Activation of adenosine A2A receptor facilitates brain-derived neurotrophic factor modulation of synaptic transmission in hippocampal slices. J Neurosci 24: 2905–2913.

Diógenes MJ, Assaífe-Lopes N, Pinto-Duarte A, Ribeiro JA, Sebastião AM (2007). Influence of age on BDNF modulation of hippocampal synaptic transmission: interplay with adenosine A2A receptors. Hippocampus 17: 577–585.

Diógenes MJ, Dias RB, Rombo DM, Vicente Miranda H, Maiolino F, Guerreiro P et al. (2012). Extracellular alpha-synuclein oligomers modulate synaptic transmission and impair LTP via NMDA-receptor activation. J Neurosci 32: 11750–11762.

Doherty FC, Sladek CD (2011). NMDA receptor subunit expression in the supraoptic nucleus of adult rats: dominance of NR2B and NR2D. Brain Res 1388: 89–99.

Dunwiddie TV, Masino SA (2001). The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24: 31–55.
Fernandes CC, Pinto-Duarte A, Ribeiro JA, Sebastião AM (2008). Postsynaptic action of brain-derived neurotrophic factor attenuates alpha4 nicotinic acetylcholine receptor-mediated responses in hippocampal interneurons. J Neurosci 28: 5611–5618.

Ferreira DG, Temido-Ferreira M, Miranda HV, Batalha VL, Coelho JE, Szigó EM et al. (2017). α-Synuclein interacts with PrPC to induce cognitive impairment through mGluR5 and NMDAR2B. Nat Neurosci 20: 1569–1579.

Fontinha BM, Delgado-Garcia JM, Madroñal N, Ribeiro JA, Sebastião AM, Gruart A (2009). Adenosine A2A receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice. Neuropsychopharmacology 34: 1865–1874.

Fontinha BM, Diógenes MJ, Ribeiro JA, Sebastião AM (2008). Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. Neuropharmacology 54: 924–933.

Fredholm BB, Ijzerman AP, Jacobson KA, Linden J, Müller CE (2011). International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors – an update. Pharmacol Rev 63: 1–34.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 46: D1091–D1106.

Hardingham GE, Bading H (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11: 682–696.

Hardingham GE, Fukunaga Y, Bading H (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 5: 405–414.

Hothersall JD, Sunil Sarda DG, Sheppard RJ, Chen H, Keur W, Waring MJ et al. (2017). Structure-activity relationships of the sustained effects of adenosine A2A receptor agonists driven by slow dissociation kinetics. Mol Pharmacol 91: 25–38.

Hu Q, Ren X, Liu Y, Li Z, Zhang L, Chen X et al. (2016). Aberrant adenosine A2A receptor signaling contributes to neurodegeneration and cognitive impairments in a mouse model of synucleinopathy. Exp Neurol 283: 213–223.

Hunt DL, Castillo PE (2012). Synaptic plasticity of NMDA receptors: mechanisms and functional implications. Curr Opin Neurobiol 22: 496–508.

Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills, Williams M (1989). [3H]CGS21680, a selective A2 adenosine receptor agonist directly labels A2 receptors in rat brain. J Pharmacol Exp Ther 251: 888–893.

Jones PA, Smith RA, Stone TW (1998). Protection against and cognitive impairments in a mouse model of synucleinopathy. Adenosine A2A receptor signaling contributes to neurodegeneration. Hu Q, Ren X, Liu Y, Zhang L, Chen X et al. Brain Res 800: 328–335.

Kadowaki-Horita T, Kobayashi M, Mori A, Jenner P, Kanda T (2013). Effects of the adenosine A2A antagonist irinoceline on cognitive performance in rats with a 6-OHDA lesion in prefrontal cortex. Psychopharmacology (Berl) 230: 345–352.

Kaster MP, Machado NJ, Silva HB, Nunes A, Ardais AP, Santana M et al. (2015). Caffeine acts through neuronal adenosine A2A receptors to prevent mood and memory dysfunction triggered by chronic stress. Proc Natl Acad Sci 112: 7833–7838.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kouvaros S, Papatheodoropoulos C (2016). Major dorsoventral differences in the modulation of the local CA1 hippocampal network by NMDA, mGlu5, adenosine A2A and cannabinoid CB1 receptors. Neuroscience 317: 47–64.

Khosravani H, Zhang Y, Tsutsui S, Hamed S, Altier C, Hamid J et al. (2008). Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. J Cell Biol 181: 551–565.

Krania F, Dimou E, Bantouna M, Kouvaros S, Tsiamaki E, Papatheodoropoulos C et al. (2018). Adenosine A2A receptors are required for Glutamate mGluR5- and Dopamine D1 receptor-evoked ERK1/2 phosphorylation in rat Hippocampus: involvement of NMDA receptor. J Neurochem 145: 217–231.

Laprell L, Repak E, Franckevicius V, Hartrampf F, Terhag J, Hollmann M et al. (2015). Optical control of NMDA receptors with a diffusible photoswitch. Nat Commun 6: 8076.

Lau CG, Znik RG (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci 8: 413–426.

Laurent C, Burnouf S, Ferry B, Batalha VL, Coelho JH, Baquin Y et al. (2016). A2A adenosine receptor deletion is protective in a mouse model of Tauopathy. Mol Psychiatry 21: 149.

Li P, Rial D, Canas PM, Yoo J-H, Li W, Zhou X et al. (2015a). Optogenetic activation of intracellular adenosine A2A receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. Mol Psychiatry 20: 1339–1349.

Li W, Silva HB, Real J, Wang Y-M, Rial D, Li P et al. (2015b). Inactivation of adenosine A2A receptors reverses working memory deficits at early stages of Huntington’s disease models. Neurobiol Dis 79: 70–80.

Lohse MJ, Nikolaev VO, Hein P, Hoffmann C, Viladara J-P, Bunemann M (2008). Optical techniques to analyse real-time activation and signalling of G-protein-coupled receptors. Trends Pharmacol Sci 29: 159–165.

Lopes LV, Cunha RA, Kull B, Fredholm BB, Ribeiro JA (2002). Adenosine A(2A) receptor facilitation of hippocampal synaptic transmission is dependent on tonic A(1) receptor inhibition. Proc Natl Acad Sci U S A 99: 1874–1879.

Lond B Biol Sci 358: 773–786.

Morris RGM, Moser EI, Biedel G, Martin SJ, Sandin J, Day M, et al. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Trans R Soc Lond B Biol Sci 358: 773–786.

Papouin T, Ladépêche L, Jérôme R, Sacchi S, Labasque M, Hanini M et al. (2012). Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150: 633–646.

Pinho J, Vale R, Batalha VL, Coelho JH, Baquin Y et al. (2016). A2A adenosine receptor deletion is protective in a mouse model of Tauopathy. Mol Psychiatry 21: 149.

Laurent C, Burnouf S, Ferry B, Batalha VL, Coelho JH, Baquin Y et al. (2016). A2A adenosine receptor deletion is protective in a mouse model of Tauopathy. Mol Psychiatry 21: 149.

Lopes LV, Cunha RA, Kull B, Fredholm BB, Ribeiro JA (2002). Adenosine A(2A) receptor facilitation of hippocampal synaptic transmission is dependent on tonic A(1) receptor inhibition. Neuroscience 112: 319–329.

Malenka RC, Nicoll RA (1999). Long-term potentiation – a decade of progress? Science 285: 1870–1874.

Morris RGM, Moser EI, Biedel G, Martin SJ, Sandin J, Day M, et al. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Trans R Soc Lond B Biol Sci 358: 773–786.

Papouin T, Ladépêche L, Jérôme R, Sacchi S, Labasque M, Hanini M et al. (2012). Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150: 633–646.

Pinho J, Vale R, Batalha VL, Costenla AR, Dias RB, Rombo DM et al. (2012). Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150: 633–646.

Ribeiro FF, Xapelli S, Miranda-Lourenço C, Tanqueiro SR, Fonseca-Gomes J, Diógenes MJ (2016). Purine nucleosides in neuroregeneration and neuroprotection. Neuropharmacology 104: 413–426.

Rebola N, Lujan R, Cunha RA, Muller C (2008). Adenosine A2A receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. Neuron 57: 121–134.

Rombo DM, Newton K, Nissen W, Badurek S, Horn JM, Minichiello L (2015). Sympathetic mechanisms of adenosine A2A...
receptor-mediated hyperexcitability in the hippocampus. Hippocampus 25: 566–580.

Sarantis K, Tsiamaki E, Kouvaros S, Papatheodoropoulos C, Angelatou F (2015). Adenosine A2A receptors permit mGluR5-evoked tyrosine phosphorylation of NR2B (Tyr1472) in rat hippocampus: a possible key mechanism in NMDA receptor modulation. J Neurochem 135: 714–726.

Sebastião AM, Ribeiro JA (2009). Tuning and fine-tuning synapses with adenosine. Curr Neuropharmacol 7: 180–194.

Sebastião AM, Ribeiro JA (2015). Neuromodulation and metamodulation by adenosine: impact and subtleties upon synaptic plasticity regulation. Brain Res 1621: 102–113.

Tanqueiro SR, Ramalho RM, Rodrigues TM, Lopes LV, Sebastião AM, Diógenes AM (2018). Inhibition of NMDA receptors prevents the loss of BDNF function induced by amyloid β. Front Pharmacol 9: 237. https://doi.org/10.3389/fphar.2018.00237, eCollection.

Tebano MT, Martire A, Rebola N, Pepponi R, Domenici MR, Grò MC et al. (2005). Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: a possible key mechanism in the modulation of N-methyl-D-aspartate effects. J Neurochem 95: 1188–1200.

Viana da Silva S, Haberl MG, Zhang P, Bethge P, Lemos C, Gonçalves N et al. (2016). Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A2A receptors. Nat Commun 7: 11915.

Temido-Ferreira M, Ferreira DG, Batalha VL, Marques-Morgado I, Coelho JE, Pereira P et al. (2018). Lopes LV (2018) age-related shift in LTD is dependent on neuronal adenosine A2A receptors interplay with mGluR5 and NMDA receptors. Mol Psychiatry. https://doi.org/10.1038/s41380-018-0110-9. [Epub ahead of print].

Wirkner K, Gerevich Z, Krause T, Gönther A, Köles L, Schneider D et al. (2004). Adenosine A2A receptor-induced inhibition of NMDA and GABA A receptor-mediated synaptic currents in a subpopulation of rat striatal neurons. Neuropharmacology 46: 994–1007.

Xia P, Chen HV, Zhang D, Lipton SA (2010). Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. J Neurosci 30: 11246–11250.

Zocchi C, Ongini E, Conti A, Monopoli A, Negretti A, Baraldi PG et al. (1996). The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A2A adenosine receptor antagonist. J Pharmacol Exp Ther 276: 398–404.