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RESEARCH PAPER

Adenosine A_{2A} receptors facilitate synaptic NMDA currents in CA1 pyramidal neurons

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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 A_{2A} subtype can influence both synaptic plasticity and neuronal death. The present work was designed to evaluate the influence of adenosine A_{2A} 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 A_{2A} 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 A_{2A} 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. A_{2A} 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 A_{2A} 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]-5′*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

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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 **A_1 receptors** are responsible for the adenosine-mediated inhibitory effects, including inhibition of neurotransmitter release and changes in postsynaptic membrane conductance (Dunwiddie 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 **A_{2A} receptor**, is coupled to Gs proteins, classically leading to stimulation of adenylate cyclase, a rise in intracellular **cAMP** levels and PKA activation. A_{2A} 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 A_{2A} 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 (Diógenes *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 A_{2A} 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 A_{2A} 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 (**$mGlu_5$** 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 A_{2A} receptor overexpression (Temido-Ferreira *et al.,* 2018). A_{2A} 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 A_{2A} receptors upon LTP and excitotoxicity, no information so far exists on the direct influence of A_{2A} 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 A_{2A} 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 A_{2A} 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; $CaCl_2$ 0.5; $MgCl_2$ 7; $NaHCO_3$ 25; NaH_2PO_4 1.25; glucose 7, bubbled with 95% O_2 and 5% CO_2, pH 7.4. Slices were first incubated for 30 min at 35°C in artificial CSF (aCSF) containing (mM): NaCl 124; KCl 3; NaH_2PO_4 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; $CaCl_2$ 2 and glucose 10, pH 7.4, gassed with 95% O_2 and 5% CO_2;

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 Mg^{2+} 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 Mg^{2+}) 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-gluconate 125; KCl 11; $CaCl_2$ 0.1; $MgCl_2$ 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 nulled 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 ψ, 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, **tetrodoxin 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 A_{2A} receptor agonist), **SCH 58261** (a selective and competitive A_{2A} 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-propylenglycol 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),

Table 1

Comparison between the effect of the A_{2A} 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 archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

Results

Activation of adenosine A_{2A} receptors facilitates NMDA-PSCs in CA1 pyramidal neurons

To study the actions of A_{2A} 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 A_{2A} agonist, CGS 21680, was applied at a concentration (30 nM) selective for A_{2A} 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 A_{2A} agonist upon NMDA-PSCs recorded from slices from 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 A_{2A} receptor activation, we tested if this action could be prevented by the selective A_{2A} 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 A_{2A} 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 A_{2A} receptor activation with CGS 21680 can also facilitate AMPA receptor-mediated currents, enhancing surface expression of Glu_1 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 A_{2A} receptors upon NMDA receptor activity in CA1 pyramidal cells does not occur exclusively through extrasynaptic NMDA receptors.

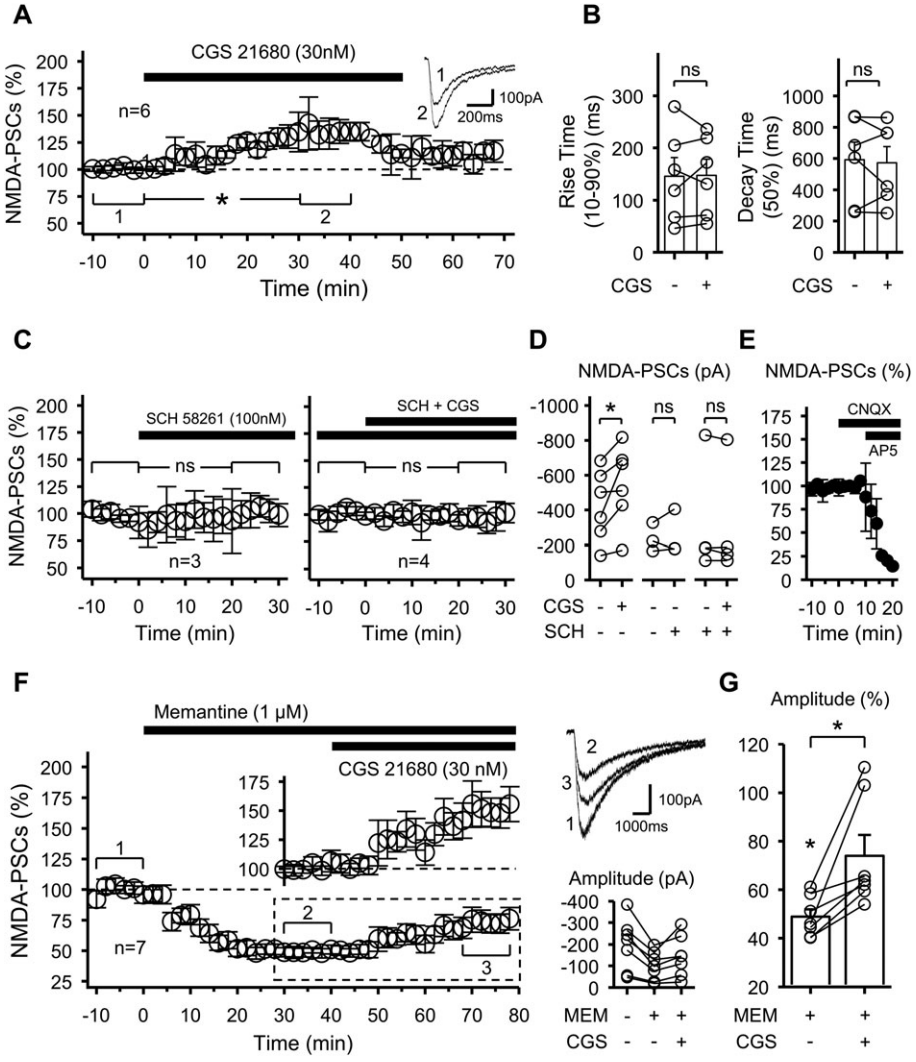

Figure 1

A_{2A} 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 A_{2A} agonist, on chemically-evoked NMDA-PSCs peak amplitude ($n = 6$, $N = 6$). Zero time in the abscissae corresponds to the time where the first 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 A_{2A} 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 A_{2A} 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 recorded from slices from 5- to 7- or 8- to 10-week-old rats did not differ significantly.

CGS 21680 may act at presynaptic A_{2A} receptors to enhance glutamate release from Schaffer collaterals with impact upon AMPA receptor-mediated synaptic transmission (Lopes *et al.*, 2002; Dias *et al.*, 2012). We thus assessed paired pulse ratios (PPR) to understand whether a presynaptic influence of A_{2A} receptor could impact upon the NMDA receptor-mediated synaptic currents. The paired-pulse ratio (PPR = 2nd $EPSC_{amplitude}$/1st $EPSC_{amplitude}$) of two consecutive NMDA-EPSCs 100 ms apart was analysed, and no significant alterations were detected in the presence of CGS 21680 (99 ± 6.8% of baseline; Figure 2A,B), indicating that A_{2A} receptor's action on NMDA-EPSCs does not result from enhanced release of glutamate.

The CGS 21680 effect was not accompanied by changes in NMDA-EPSCs kinetics as evidenced by unaltered 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 synaptically 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 21680; Figure 3A,B) or current kinetics

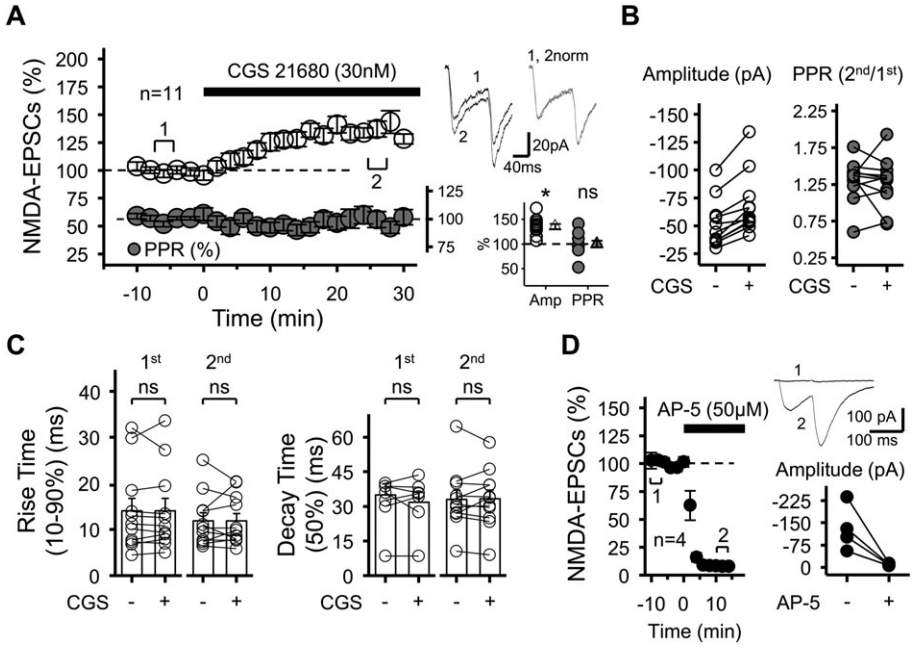

Figure 2

Adenosine A_{2A} receptor activation potentiates afferent-evoked NMDA receptor-mediated currents (NMDA-EPSCs). (A) Average normalized time course changes in afferent-evoked NMDA-EPSCs amplitude (first pulse amplitude, open symbols) and PPR (second vs. first pulse amplitude) induced by superfusion of selective A_{2A} receptor agonist, CGS 21680 (30 nM) (horizontal bar). Zero time in the abscissae corresponds to the time where the first test solution started to be perfused. Data are presented as mean ± SEM (n = 11, N = 6). *Right upper inset*: sample traces (average of eight consecutive currents, 2 min) taken from a representative experiment; before (1) and 30 min after CGS 21680 (2) superfusion; trace showing 30 min after CGS 21680 superfusion was normalized for baseline trace (1 + 2norm) to point out the PPR; the stimulus artefact for each trace was removed for better visualization. *Right bottom panel*: summary plot showing baseline-normalized NMDA-EPSCs peak amplitude (first pulse amplitude, Amp) and PPR (second vs. first pulse amplitude) following addition of CGS 21680. Circles correspond to individual experiments; triangles represent mean ± SEM. *$P < 0.05$; ns, not significant (n = 11, N = 6, paired t-test). (B) Summary plot showing NMDA-EPSC peak amplitude (first pulse amplitude, pA) (left panel) and PPR (second vs. first pulse amplitude) (right panel) of all individual cells shown in (A). (C) 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 first and second NMDA-EPSC pulses of each individual experiment, with a line connecting values at baseline and after 30 min of CGS 21680 perfusion. Histograms depict mean ± SEM for each set of values. ns, not significant [rise time first pulse, n = 11, P = 0.95; rise time second pulse, n = 11, P = 0.98; decay time second pulse, n = 11, P = 0.84 (Student's paired t-test) and decay time first pulse, n = 7, P = 0.81, Wilcoxon matched-pairs test]. (D) Averaged normalized time course changes of the effect of DL-AP5 (50 μM) (horizontal bar) on NMDA-EPSC peak amplitude. *Right upper inset*: sample traces (average of eight consecutive currents, 2 min) taken from a representative experiment before (1) and 20 min after DL-AP5 (2) superfusion; the stimulus artefact for each trace was removed for better visualization. *Right bottom panel*: summary plot showing NMDA-EPSC peak amplitude (first pulse amplitude, pA) of all individual cells shown in right panel (3: baseline; 4, DL-AP5).

(Table 2), indicating the lack of A_{2A} 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 A_{2A} 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 A_{2A} 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 A_{2A} 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 A_{2A} receptors, the cAMP/PKA signalling cascade, in the modulating effect of A_{2A} receptors on NMDA receptors.

Altogether, our results strongly indicate a preferential action of A_{2A} receptors on synaptic and not extrasynaptic NMDA receptors *via* cAMP/PKA signalling.

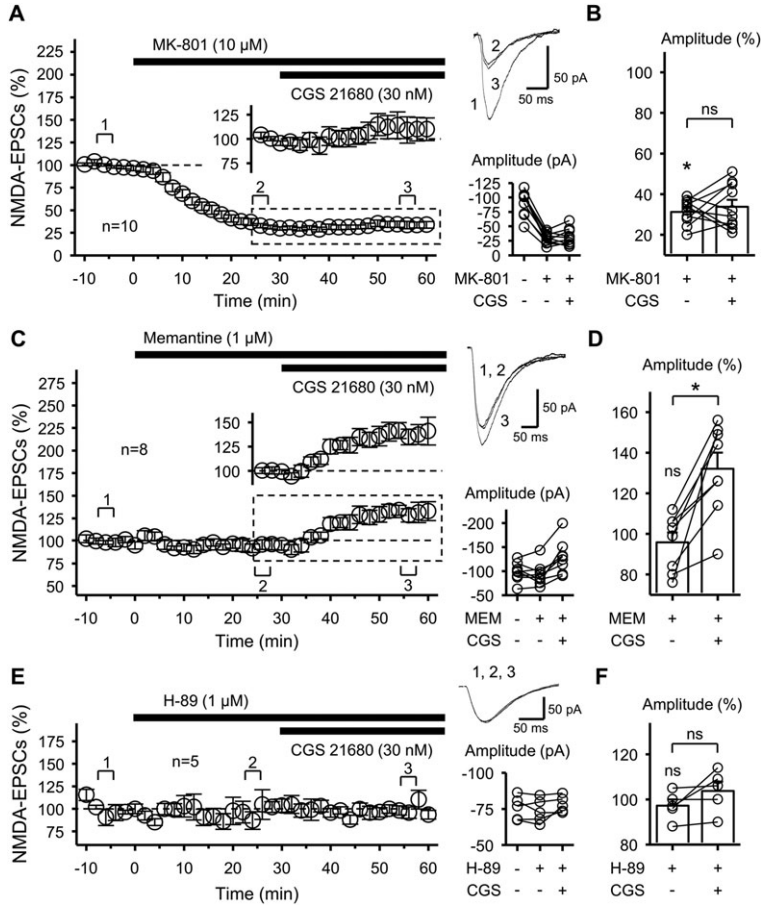

Figure 3

Synaptic, but not extrasynaptic, NMDA-EPSCs are affected by adenosine A_{2A} 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 A_{2A} receptor agonist, bottom horizontal bar), on electrically-evoked NMDA-EPSCs peak amplitude ($n = 10$, $N = 5$). Zero time in the abscissae 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 A_{2A} 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), memantine 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 A_{2A} 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 + CG S21680 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 neuromodulators, 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 gyrys-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} receptor in heathy 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.

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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, Faccenda 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, Faccenda 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, Shpiro N, Hastie CJ, Mclauchlan H *et al*. (2007). The selectivity of protein kinase inhibitors: a further update. Biochem J 408: 297–315.

Canas PM, Porciuncula 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 A 2A 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 A_{2A} 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 [^{3}H] GABA release from rat hippocampal nerve terminals. Neuropharmachology 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, Ahluwalia A, Alexander SPA, Giembycz MA *et al*. (2015). Experimental design andanalysis 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 A_1 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 A_{2A} 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, Assaife-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 alpha7 nicotinic acetylcholine receptor-mediated responses in hippocampal interneurons. J Neurosci 28: 5611–5618.

Ferreira DG, Temido-Ferreira M, Miranda HV, Batalha VL, Coelho JE, Szegö ÉM *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 A(2A) 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 hippocampal kainate excitotoxicity by intracerebral administration of an adenosine A(2A) receptor antagonist. Brain Res 800: 328–335.

Kadowaki-Horita T, Kobayashi M, Mori A, Jenner P, Kanda T (2013). Effects of the adenosine A2A antagonist istradefylline 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, Hameed S, Altier C, Hamid J *et al*. (2008). Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. J Cell Biol 181: 551–565.

Krania P, 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, Zukin RS (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 JE, Baqin 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, Vilardaga 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. Neuroscience 112: 319–329.

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

Morris RGM, Moser EI, Riedel 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*. (2017). Enhanced LTP in aged rats: detrimental or compensatory? Neuropharmacology 114: 12–19.

Ribeiro FF, Xapelli S, Miranda-Lourenço C, Tanqueiro SR, Fonseca-Gomes J, Diógenes MJ (2016). Purine nucleosides in neuroregeneration and neuroprotection. Neuropharmacology 104: 226–242.

Rebola N, Lujan R, Cunha RA, Mulle 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). Synaptic 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, Günther 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.