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Inactivation of adenosine A_{2A} receptors reverses working memory deficits at early stages of Huntington's disease models

Running title: A_{2A} receptors control cognition in HD

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Abstract

Cognitive impairments in Huntington's disease (HD) are attributed to a dysfunction of the cortico-striatal pathway and significantly affect the quality of life of the patients, but this has not been a therapeutic focus in HD to date. We postulated that adenosine A_{2A} receptors ($A_{2A}R$), located at pre- and post-synaptic elements of the cortico-striatal pathways, modulate striatal neurotransmission and synaptic plasticity and cognitive behaviors. To critically evaluate the ability of $A_{2A}R$ inactivation to prevent cognitive deficits in early HD, we cross-bred $A_{2A}R$ knockout (KO) mice with two R6/2 transgenic lines of HD (CAG120 and CAG240) to generate two double transgenic R6/2-CAG120- $A_{2A}R$ KO and R6/2-CAG240- $A_{2A}R$ KO mice and their corresponding wild-type (WT) littermates. Genetic inactivation of $A_{2A}R$ prevented working memory deficits induced by R6/2-CAG120 at postnatal week 6 and by R6/2-CAG240 at postnatal month 2 and postnatal month 3, without modifying motor deficits. Similarly the $A_{2A}R$ antagonist KW6002 selectively reverted working memory deficits in R6/2-CAG240 mice at postnatal month 3. The search for possible mechanisms indicated that the genetic inactivation of $A_{2A}R$ did not affect ubiquitin-positive neuronal inclusions, astrogliosis or Thr-75 phosphorylation of DARPP-32 in the striatum. Importantly, $A_{2A}R$ blockade preferentially controlled long-term depression at cortico-striatal synapses in R6/2-CAG240 at postnatal week 6. The reported reversal of working memory deficits in R6/2 mice by the genetic and pharmacological inactivation of $A_{2A}R$ provides a proof-of-principle for $A_{2A}R$ as novel targets to reverse cognitive deficits in HD, likely by controlling LTD deregulation.

Key words: adenosine A_{2A} receptor, Huntington's disease, cognition, working memory, long-term depression, R6/2 mice

Abbreviations: $A_{2A}R$ = adenosine A_{2A} receptor; aCSF= artificial cerebrospinal fluid; DARPP-32= dopamine- and cAMP-regulated phosphoprotein with molecular weight of 32 kDa; HD= Huntington's disease; KO= knock out; LTD= long-term depression; LTP= long-term potentiation; PN= postnatal day; WT= wild type.

Introduction

Huntington's disease (HD) is characterized by progressive motor, cognitive, and psychiatric symptoms (Zuccato et al., 2010). The largest and most comprehensive study of HD cognitive impairment (PREDICT-HD study, including 738 HD prodromes) (Stout et al., 2011) and several others (Duff et al., 2010; Lawrence et al., 1998; Papp et al., 2011) have conclusively demonstrated cognitive impairments in the HD prodrome, 5-15 years before the onset of motor symptoms. Cognitive impairment in early HD patients is characterized by perseverative behavior, reduced reversal learning and working memory deficits (Giralt et al., 2012; Papp et al., 2011). Similarly, transgenic HD (R6/2 and YAC128) (Brooks et al., 2006; Brooks et al., 2012; Lione et al., 1999; Mazarakis et al., 2005; Van Raamsdonk et al., 2005) and knock-in (KI) mouse lines (Brooks et al., 2006; Trueman et al., 2009) display impairments of reversal learning and working memory. These cognitive changes herald a primary dysfunction of the cortico-striatal pathway (Cepeda et al., 2010; Cepeda et al., 2007), and significantly affect the patient's quality of life, but this has not been a therapeutic focus in HD to date. We postulate that striatal adenosine A_{2A} receptors ($A_{2A}R$) at pre- and post-synaptic elements of cortico-striatal synapses control cortico-striatal function and reverse cognitive inflexibility in early HD. While *presynaptic* $A_{2A}R$ at corticostriatal glutamatergic terminals (Rebola et al., 2005a; Rosin et al., 2003) strategically modulate glutamate release (Ciruela et al., 2006; Rodrigues et al., 2005), *postsynaptic* striatal $A_{2A}R$ physically and functionally interact antagonistically with dopamine D_2 and NMDA receptors and synergistically with metabotropic glutamate 5 and cannabinoid CB1 receptors (reviewed in Schiffmann et al., 2007). $A_{2A}R$ at the corticostriatal pathway can fine-tune striatal synaptic plasticity by modulating long-term plasticity at cortico-striatal synapses (d'Alcantara et al., 2001; Flajolet et al., 2008; Shen et al., 2008), thus **potentially** controlling the glutamatergic hyperactivity at cortico-striatal synapses **in early HD** (Hong et al., 2012; Joshi et al., 2009; Klapstein et al., 2001). Consistent with the ability of $A_{2A}R$ to control striatal plasticity, we have shown that genetic inactivation of $A_{2A}R$ enhances working memory (Augusto et al., 2013; Wei et al., 2011; Zhou et al., 2009), reversal learning (Wei et al., 2011) and goal-oriented behavior (Yu et al., 2009). Furthermore, pharmacological blockade of $A_{2A}R$ prevents memory impairment in models of Alzheimer's disease (Canas et al., 2009; Cunha et al., 2008),

Parkinson's disease (Kadowaki Horita et al., 2013) and aging (Prediger et al., 2005). Thus, A_{2A}R emerge as promising therapeutic targets to reverse cognitive deficits in HD.

To critically evaluate the ability of A_{2A}R inactivation to reverse cognitive deficits in HD, we cross-bred A_{2A}R knockout (KO) mice with two R6/2 transgenic lines of HD (CAG120 and CAG240) to generate two double transgenic R6/2-CAG120-A_{2A}R KO and R6/2-CAG240-A_{2A}R KO mice. We focused on the ability of A_{2A}R inactivation to reverse cognitive deficit (specifically working memory) and synaptic plasticity at early stages of HD, making it a more plausible therapeutic strategy without the need to consider reversing striatal neurodegeneration.

Materials and methods

Generation of two double transgenic R6/2-CAG120 or R6/2-CAG240 x A_{2A}R KO lines

Double transgenic R6/2-A_{2A}R KO mice were generated by crossing congenic global-A_{2A}R KO mice in C57BL/6 background (Chen et al., 1999) with R6/2-CAG120 or with R6/2-CAG240 mice. We first cross-bred A_{2A}R KO with R6/2-CAG120 lines (in mixed C57BL6 x B6CBA genetic background) taking advantage of the clear experimental endpoints provided by this R6/2-CAG120 line (Gil and Rego, 2009; Menalled and Chesselet, 2002). Thus, R6/2-CAG120 mice develop cognitive deficits starting at post-natal day (PN) 35, prior to the onset of locomotor deficits, which are evident around PN40 (Carter et al., 1999; Lione et al., 1999). Since this provides a short window to study cognitive deficits without motor confounding effects, we next explored R6/2-CAG240 mice (in congenic C57BL6 genetic background), which exhibits cognitive deficits at postnatal month 2, prior to motor symptoms. Importantly, we have adapted an ovarian transplant strategy to increase our ability to generate sufficient large cohorts of the double transgenic lines. Animals were maintained in a controlled environment (23±2°C; 12 h light/dark cycle; *ad libitum* access to food and water). All studies and manipulations of animals were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, FELASA and ARRIVE and were authorized by the Animal Care and Use Committee at Boston University School of Medicine and the Ethical Committee of the Center for Neuroscience and Cell Biology of the

University of Coimbra.

Treatment with the A_{2A}R antagonist KW6002

KW6002 was synthesized by Christa Muller's lab (Bonn University, Germany), as previously described (Hockemeyer et al., 2004). KW6002 was dissolved in a fresh mixture of dimethylsulfoxide (15%) and ethoxylated castor oil (15%; Alkamuls, EL-620, Rhodia) and was injected (10 mL/kg) intra-peritoneally at a dose of 1 mg/kg, 30 min prior to behavioral testing.

Behavioral analyses

Motor coordination: After familiarization to the rotarod apparatus (Columbus Instruments) during 2 day, mice were placed on a rod that was rotated at accelerating speed from 4-40 rpm. Each mouse was given three trials for a maximum of 300 sec for each trial. The time taken by each mouse to fall off the rotating rod was recorded and used as an index of motor coordination.

Reference and working memory: We evaluated working memory and short-term reference using the 8-arm radial maze, as previously described (Augusto et al., 2013; Zhou et al., 2009). The 8-arm radial maze consists of an octagonal platform and eight radial arms, which were extending outwards from the platform (San Diego Instrument). First, a food deprivation schedule was carried out in order to reduce the animal's weight to 85% of the baseline. Next, an acclimation trial was conducted: on day 1, food pellets were scattered throughout the arms and platform, and mice were allowed to freely eat the food in the maze; on day 2, food pellets were placed in the arms only; on day 3, food pellets were located on the food cup. We then started the testing period in the next 5 days. Each day, food pellets were placed in the cups of only 4 arms (e.g. arms #1, #2, #4 and #7) while the remaining 4 arms were empty. At the beginning of each trial, the mouse was placed in the central platform and allowed to explore the maze. When the four food pellets were eaten or 5 min is elapsed, a trial was completed. A working memory error is defined when an animal revisits an arm of the maze that was just entered in the course of collecting all 4 rewards while a reference memory error is defined when the animal visits a non-baited arm (arms #3, #5, #6 and #8).

Because some of the mice failed to eat all of the food pellets on the first testing day (day 1), we recorded the mouse performance from the second testing day (day 2).

To exclude the possible confounding effect of alterations of locomotor activity after KW6002 treatment (1 mg/kg), we used the frequency of entrance into the arms (entrances/10 seconds) as an indirect indicator of the general locomotor activity.

Survival analysis: Mice were observed twice daily, in the morning and later afternoon, and weighted twice a week at the same time. The criteria for euthanasia were: (1) decrease of body weight of more than 20% within one week, (2) inability to obtain food and water, (3) difficulties in breathing and inability to move. These end points were considered equivalent to the time of death.

Immunohistochemistry analyses

At the end of behavioral testing, the mice were anesthetized with Avertin and perfused transcardially with ice-cold 0.9% saline followed by 4% paraformaldehyde. Brains were post-fixed for 1 h, rinsed in 0.1 M phosphate buffered saline, and then cryoprotected in a graded series of 10% and 20% glycerol with 2% dimethylsulfoxide solution. Frozen brains were sectioned at 30 μ m using a sledge microtome. The sections were processed for immunohistochemistry of astrogliosis (GFAP immunoreactivity), ubiquitin-positive neuronal intra-nuclear inclusions and DARPP-32 phosphorylation at Thr-75 focusing in the striatum, as previously described (Rebola et al., 2011; Shen et al., 2013), to correlate these neurochemical changes with the working memory deficits in R6/2 mice and their reversal by A_{2A}R inactivation. Free-floating sections were incubated for 30 min in 0.3% Triton-X100 and 3% normal donkey or goat serum. Incubations with primary antibodies against glial fibrillary acidic protein (GFAP, Millipore, 1:1000), ubiquitin (Dako, 1:100), or DARPP-32-phospho-threonine75 (Cell Signal, 1:1000) were conducted overnight in the presence of 0.01% Triton-X100 and 3% normal donkey serum. After washing, the sections were incubated for 1 h at room temperature with AlexaFluor 488- or AlexaFluor 594-conjugated secondary antibodies (Molecular Probes, 1:200), washed and mounted on slides with vectashield (Vector Labs) and images were acquired with a fluorescence microscope.

Radioligand binding assays in nerve terminal membranes

Nerve terminals from the striatum of male R6/2-CAG120 [B6CBA-Tg(HDexon1)62Gpb/3J from Jackson Laboratories] with 35-40 days were purified using combined sucrose and Percoll fractionation, as previously described (Rodrigues et al., 2008). The purity of the nerve terminals was confirmed by immunocytochemistry since over 95% of visualized elements were immunopositive for synaptophysin and less than 5% were immunopositive for GFAP or PSD95, i.e. they mostly corresponded to the presynaptic component of synapses (Rodrigues et al., 2008). The density of A_{2A}R was estimated with a radioligand binding assay using a supra-maximal concentration of the A_{2A}R antagonist [³H]SCH58261 (6 nM; offered by Dr. E.Ongini, Schering-Plough, Italy), as previously described (Lopes et al., 2004). Binding reactions were for 1 h at room temperature (23±1°C) with 24-36 µg of protein in a final volume of 200 µL of a Tris-Mg solution (50 mM Tris and 2 mM MgCl₂ pH 7.4) and 4 U/mL of adenosine deaminase, with constant swirling. Specific binding was determined by subtraction of non-specific binding, measured in the presence of 3 µM XAC (Tocris, Bristol, UK), a mixed A₁R/A₂R antagonist. Binding reactions were stopped by addition of 4 mL of ice-cold Tris-Mg solution and filtration through Whatman GF/C glass microfiber filters (GE Healthcare) in a filtration system (Millipore). The radioactivity was measured at least 12 hours after adding 2 mL of scintillation liquid (AquaSafe 500Plus, Zinsser) in a Tricarb β-counter (Perkin Elmer). All these binding assays were performed in triplicate and the values are presented as mean±S.E.M. of n=3-4 (i.e. in preparation obtained from different mice) and expressed as fmol/mg protein.

Synaptic plasticity at cortico-striatal synapses

Electrophysiological recordings of cortico-striatal transmission were carried out essentially as previously described (Quiroz et al., 2009). Coronal cortico-striatal slices (400 µm thick) were prepared from male R6/2-CAG120 [B6CBA-Tg(HDexon1)62Gpb/3J from Jackson Laboratories] with 35-40 days, and allowed to equilibrate for at least 90 min at room temperature in modified artificial cerebrospinal fluid (aCSF) containing (in mM); 124 NaCl, 4.5 KCl, 2 CaCl₂, 1 MgCl₂, 26

NaHCO₃, 1.2 NaH₂PO₄ and 10 D-glucose, bubbled with a gas mixture of 95% O₂ / 5% CO₂. One slice was transferred to a submerged recording chamber and continuously superfused with gassed aCSF at a flow rate of 3 mL/min. Stimulation was delivered at a frequency of 0.05 Hz through a bipolar twisted tungsten wire placed in the white matter above the dorsolateral striatum. Extracellular field recordings were obtained with micropipettes (2–4 MΩ) filled with 4 M NaCl, placed in the dorsolateral striatum, to record population spike responses to estimate synaptic efficacy. Recordings were obtained with an ISO-80 amplifier (World Precision Instruments) and digitized using an ADC-42 board (Pico Technologies). Averages of 4 consecutive responses were continuously monitored on a personal computer with the LTP 1.01 software (Anderson and Collingridge, 2001).

Synaptic plasticity was triggered by frequency stimulation trains, applied at least 30 min after obtaining a stable baseline: to trigger long-term potentiation (LTP), we applied three high frequency stimulation trains of 100 Hz for 1 second each delivered every 5 seconds, whereas to trigger long-term depression (LTD), we applied a low frequency train of 10 Hz for 10 min. The amplitude of synaptic plasticity (LTP or LTD) was quantified as the percentage change between two values: the average amplitude of the five potentials taken between 35 and 45 min after the end of the induction protocol in relation to the average amplitude of the field potentials measured during 10 min that preceded the induction protocol.

To test the role of A_{2A}R on synaptic plasticity, we used the selective antagonist SCH58261 {7-(2- phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazol-ol[1,5c]pyrimidine; from Tocris} at a selective but supra-maximal concentration of 50 nM (Lopes et al., 2004). SCH58261 was made up as a 5 mM stock solution in dimethylsulfoxide, and dissolved in aCSF solution to a concentration of 50 nM.

Statistical Analysis

Single statistical comparisons between two groups were analyzed using a non-paired two-tailed Student's *t* test. Statistical comparison of behavioral parameters at multiple time points was

analyzed by repeated measure ANOVA or one-way ANOVA followed by a Tukey's multiple-comparison post hoc test or by a Dunnett's multiple-comparison post hoc test (for comparison with specific controls). Data with more than one variable and/or condition were analyzed with a two-way ANOVA followed by Bonferroni post hoc tests. Survival data were analyzed using the Mantel-cox log-rank test and Kaplan-Meier survival curves. Unless otherwise indicated, the significance level was 95%.

Results

Genetic inactivation of $A_{2A}R$ reversed working memory deficit in R6/2 mice

We focused on working memory, which is defined as the capacity to maintain information held "online" to select appropriate behavioral responses (Goldman-Rakic, 1996), for three reasons: i) it is typically impaired in HD models; ii) it is associated with a dysfunction of the cortico-striatal pathway; iii) it is modulated by the striatal $A_{2A}R$ in normal animals. Using an 8-arm radial maze, we found that CAG120-WT mice at postnatal week 6 displayed significant deficits of working memory compared to WT-WT mice (Fig.1A, $n=12-15/\text{group}$). Genetic inactivation of $A_{2A}R$ selectively reduced working memory errors in normal (WT- $A_{2A}R$ KO compared to WT-WT mice) as well as in CAG120 mice (CAG120- $A_{2A}R$ KO compared to CAG120-WT) (Fig. 1A), without significant effect on reference memory (Fig. 1B). When tested in the rotarod test (Fig. 1C, $n=13-18/\text{group}$), we observed that CAG120-WT mice at postnatal week 6 displayed locomotor deficits typified by a shorter latency to fall from the rotating platform, compared to WT-WT mice. Notably, the genetic inactivation of $A_{2A}R$ did not modify the rotarod performance in WT mice (WT- $A_{2A}R$ KO compared to WT-WT mice) nor did it recover performance in CAG120 mice (CAG120- $A_{2A}R$ KO compared to CAG120-WT) (Fig. 1C). Thus genetic inactivation of $A_{2A}R$ selectively rescued working memory deficits, independently of locomotor deficits.

To overcome the difficulty in assessing cognition associated with the rapid onset of motor

deficits in the R6/2-CAG120 mice, we analyzed another R6 mouse line (R6/2-CAG240), which has a longer presymptomatic period with a delayed presentation of motor symptom and is better suited for cognitive behavioral analysis during the prodrome. Thus, CAG240-WT mice (n=12-16/group) did not display locomotor deficits in the rotarod test at postnatal month 2 (Fig. 2C), which became evident at postnatal month 3 (Fig. 2G). However, consistent with cognitive dysfunction being a characteristic of HD prodrome, CAG240-WT mice at postnatal month 2 already displayed deficits of working memory (particularly on the testing day 3; Fig. 2A) and of short-term reference memory (on the testing day 2-3; Fig. 2B), compared to WT littermates (WT-WT). These cognitive deficits were maintained upon emergence of motor deficits, since CAG240-WT mice at postnatal month 3 still displayed clear deficits of working memory (testing days 4-5; Fig. 2D) and reference memory (testing days 3-4; Fig. 2E). Importantly, the genetic inactivation of $A_{2A}R$ selectively reversed working and reference memory deficits (comparing CAG240- $A_{2A}R$ KO with CAG240-WT mice) both at postnatal month 2 (Fig. 2A, B) and at postnatal month 3 (Fig. 2D, E), without effects on locomotion tested in the rota-rod test (Fig. 2C, F).

Together, these findings show for the first time that the genetic inactivation of $A_{2A}R$ reverses the impairment of working memory in R6/2 mice of both CAG120 and CGA240 lines, independently of locomotor modifications.

Pharmacological blockade of $A_{2A}R$ also reversed working memory deficit in R6/2 mice

We next investigated if the pharmacological blockade of $A_{2A}R$ mimicked the beneficial impact of the genetic $A_{2A}R$ inactivation on the working memory deficits of R6/2 mice. As shown in Figure 3A, the treatment of R6/2-CAG240 mice at postnatal month 3 with the selective $A_{2A}R$ antagonist KW6002 (1 mg/kg) significantly ameliorated working memory performance (n=7-8). Notably, this dose of KW6002, which produces little effect on motor activity (as indicated by their entrance frequencies to the arm, Fig. 3C), did not affect reference memory in R6/2-CAG240 mice at postnatal month 3 (Fig. 3B).

Blockade of $A_{2A}R$ did not modify ubiquitin-positive neuronal inclusions, astrogliosis or

DARPP-32 activity in R6/2 mice.

To foster the mechanism underlying the ability of A_{2A}R blockade to rescue working memory deficits in HD, we carried out a qualitative screening to examine the effect of genetic A_{2A}R inactivation on some candidate mechanisms currently explored to manage HD, namely the ubiquitin-proteasome system (Seo et al., 2004), the deposition of the mutant huntingtin protein (DiFiglia et al., 1997), reactive astrogliosis (Bradford et al., 2009; Selkoe et al., 1982) and aberrant cortico-striatal transmission (Cepeda et al., 2007; Hohn et al., 2011; Hong et al., 2012).

As shown in Figure 4A, R6/2-CAG120 mice at postnatal day (PN) 70 (a relatively late stage of this line) displayed a scatter distribution pattern of ubiquitin-positive neuronal intranuclear inclusions in some areas of the cortex and striatum, which were indistinguishable between CAG120-WT and CAG120-A_{2A}R KO mice. We also examined R6/2-CAG240 mice at postnatal month 2, which did not present any ubiquitin-positive neuronal intranuclear inclusions in the cortex or striatum regardless of A_{2A}R genotype (data not shown).

We next used GFAP immunoreactivity as an index of reactive astrogliosis (Pekny and Nilsson, 2005). R6/2-CAG240 mice at postnatal month 3 displayed increased GFAP staining in the white matter between cortical and striatal regions and in the cortex (insert image from the box location in Fig. 4B), which was indistinguishable between CAG240-WT and CAG240-A_{2A}R KO mice (Fig. 4B). A similar GFAP staining was observed in CAG120 mice at PN50 and, again, there was no significant difference of GFAP immunoreactivity between R6/2-WT and R6/2-A_{2A}R KO (Fig. 4B).

To obtain a general estimate of a possible influence of the genetic deletion of A_{2A}R on cortico-striatal activity, we compared the immunoreactivity of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein) phosphorylated at threonine-75, which has been proposed as marker of the glutamatergic drive of medium spiny neurons (Nishi et al., 2005) that we previously showed to be modulated by presynaptic A_{2A}R (Shen et al., 2013). As shown in Figure 4C, immunoreactivity for DARPP-32 phosphorylation at Thr-75 (32-Thr-75-p) was largely enriched in striatum.

Importantly, DARPP-32-Thr-75-p levels were markedly reduced in R6/2-WT and WT-A_{2A}R KO compared to WT-WT. Double transgenic mice (i.e. R6/2-A_{2A}R KO) did not show any further reduction in DARPP-32-Thr-75-p compared to WT-A_{2A}R KO or R6/2-WT alone (CAG120 line at PN50).

Increased density and gain of function of A_{2A}R to control of long-term depression in R6/2 mice

We detected an increase of the binding density of ³H-SCH58261 (6 nM) in nerve terminal membranes of R6/2 mice at postnatal week 6 (953±51 fmol/mg protein, n=5) compared to WT mice (695±8 fmol/mg protein, n=5) (Fig. 4D). This prompted investigating a possible gain of function of A_{2A}R in the control of synaptic plasticity in cortico-striatal synapses, which is the most relevant neurophysiological role of A_{2A}R (Cunha, 2008) and is critically involved in reverting abnormal changes of synaptic plasticity and behavior (Batalha et al., 2013). In *ex vivo* slice electrophysiological experiments, we found that the amplitude of low frequency-induced long-term depression (LTD) in cortico-striatal synapses was similar (p<0.05) in WT and R6/2 mice (Fig. 5A, B), without changes in the input-output curves between the two groups of mice (data not shown). However, whereas the blockade of A_{2A}R with the selective antagonist SCH58261 (50 nM) did not significantly affect LTD amplitude in WT mice (Fig. 5A, C), A_{2A}R blockade significantly (p<0.05) decreased LTD amplitude in slices from R6/2 mice (Fig. 5A, D).

Interestingly, there was a selective modification of the impact of A_{2A}R in the control of LTD rather than in the control of high frequency-induced long-term potentiation (LTP) at cortico-striatal synapses (Fig. 5E-H). In fact, the amplitude of LTP was similar (p>0.05) between WT and R6/2 mice (Fig. 5E, F) and SCH58261 (50 nM) decreased LTP (p<0.05) in WT (Fig. 5E, G) and R6/2 mice (Fig. 5E, H) to a similar extent (Fig. 5E; p>0.05). This impact of A_{2A}R on synaptic plasticity contrasted with the lack of effect of SCH58261 on basal synaptic transmission (data not shown), confirming that A_{2A}R are also selectively engaged to control synaptic plasticity phenomena (Cunha, 2008) at cortico-striatal synapses.

Long-term impact of the genetic $A_{2A}R$ inactivation on the survival of R6/2 mice

In order to bolster the therapeutic interest of blocking $A_{2A}R$ to manage cognitive impairments in early HD, we next investigated if the genetic inactivation of $A_{2A}R$ caused long-term detrimental effects in R6/2 mice by investigating their survival. We report that the genetic inactivation of $A_{2A}R$ in CAG120 mice markedly increased their survival (PN134) compared to the $A_{2A}R$ WT background (PN85) (Fig. 6A). Furthermore, CAG120- $A_{2A}R$ heterozygous KO mice showed intermediate improvement (average PN99), suggesting a specific $A_{2A}R$ gene dose effect on survival. Intriguingly, we noted that this protective phenotype in R6/2-CAG120- $A_{2A}R$ KO mice appeared to be associated with an attenuated seizure activity (gauged by visual inspection), well characterized in this mouse line upon aging (Mangiarini et al., 2006): CAG120-WT developed seizure activity more frequently than CAG120- $A_{2A}R$ KO, a phenotype that might be related to the mixed genetic background (*C57BL6J* x *CBAJ*). Although this contention was based on a non-quantitative analysis, it is re-enforced by the lack of effect of $A_{2A}R$ inactivation in R6/2-CAG240 (Fig. 6B), generated in congenic C57BL6 background and which developed significantly less seizure activity (Mangiarini et al., 2006).

Discussion

By combining the use of double transgenic with $A_{2A}R$ deletion from two transgenic lines (R6/2- CAG120 and R6/2-CAG240) modeling Huntington's disease (HD), with the selective $A_{2A}R$ antagonist KW6002, we show here that $A_{2A}R$ blockade reverts the deficits of working memory both at prodrome and early phases of HD R6/2 mice, recovering the performance of control mice. We also found an up-regulation of synaptic $A_{2A}R$ in early HD and a gain of function of $A_{2A}R$ to control LTD in cortico-striatal synapses, which are aberrantly altered in early HD (e.g. Cepeda et al., 2007; Höhn et al., 2011; Hong et al., 2012) and $A_{2A}R$ antagonists reverse it. These findings prompt the exciting possibility that striatal $A_{2A}R$ may represent a novel target against the cognitive inflexibility (such as working memory impairment) in the HD prodrome (Papp et al., 2011; Stout et al., 2011).

The conclusion that A_{2A}R rescue the deficits of working memory in early HD extends to a pathological condition the striking ability of A_{2A}R to control working memory and cognitive flexibility (Augusto et al., 2013; Wei et al., 2011; Yu et al., 2009; Zhou et al., 2009). This beneficial impact resulting from A_{2A}R deletion on working memory had a qualitatively similar impact in the two R6/2 mouse lines used in the present study, which have a different genetic background, thus increasing the robustness of our present conclusion. The use of transgenic mice with cell type-selective deletions of A_{2A}R has previously enabled us to determine that it was the specific population of A_{2A}R in cortico-striatal pathways that was responsible for the control of working memory and habit formation in normal animals (Augusto et al., 2013; Wei et al., 2011; Yu et al., 2009; Zhou et al., 2009). Remarkably, several studies have shown that it is precisely cortico-striatal pathways that undergo the most precocious neurophysiological alterations that parallel cognitive impairment in the prodrome of HD (Cepeda et al., 2007; Hong et al., 2012; Joshi et al., 2009; Klapstein et al., 2001). Indeed, before the emergence of motor deficits, there is a parallel hyper-excitability (Cepeda et al., 2007; Joshi et al., 2009; Klapstein et al., 2001) and partial disconnection of the cortico-striatal pathway (Hohn et al., 2011; Hong et al., 2012) that is associated with alterations of synaptic plasticity (Dalbem et al., 2005; Kung et al., 2007). This imbalance of synaptic plasticity has been proposed to be the neurophysiological basis of striatal-dependent learning (reviewed in Lovinger et al., 2010), namely the alterations of long-term depression (LTD) (Kheirbek et al., 2009; Lovinger, 2010). We now observed that A_{2A}R selectively controlled LTD without affecting basal synaptic transmission or the glutamate-dependent phosphorylation of threonine-75 of DARPP-32 in the targeted medium spiny neurons. This simultaneous selective control of LTD in cortico-striatal pathways and working memory dysfunction in R6/2 mice upon genetic or pharmacological blockade of A_{2A}R, thus suggests that the gain of function of A_{2A}R to control striatal LTD may underlie the ability of A_{2A}R blockade to revert working memory deficits in R6/2 mice, an hypothesis that still needs additional experimental confirmation. It also remains to be determined if this ability of A_{2A}R to control striatal LTD and working memory dysfunction may involve the ability of A_{2A}R to modulate either NMDA receptors (Rebola et al., 2008; Tebano et al., 2005; Wirkner et al., 2004) that play a key role to induce striatal synaptic plasticity (e.g. Lovinger, 2010), or BDNF

bioavailability (Minghetti et al., 2007) and its impact on the expression of synaptic plasticity (reviewed in Sebastião and Ribeiro, 2009), or cannabinoid CB1 receptors (Ferré et al., 2010; Martire et al., 2011) also involved in LTD expression (Gerdeman et al., 2002), or dopamine D2 receptor function (Tozzi et al., 2007) to shift the balance of synaptic plasticity in the striatum (Shen et al., 2008). Furthermore, our present conclusion that the A_{2A}R-mediated control of cortico-striatal plasticity underlies the impact of A_{2A}R on working memory dysfunction does not exclude the possibility that there might be an additional participation of A_{2A}R controlling neuronal circuits in other brain regions (Bannon et al., 2014; Rebola et al., 2008) where abnormal information flow has also been found in association with cognitive impairments in early HD, such as the hippocampus (e.g. Milnerwood et al., 2006; Milnerwood and Raymond, 2010), prefrontal (Dallerac et al., 2011) or perirhinal cortex (Cummings et al., 2006).

This gain of function of A_{2A}R to control striatal LTD and working memory deficits in R6/2 mice was associated with an up-regulation of A_{2A}R in the presynaptic compartments in the striatum of R6/2 mice at motor presymptomatic phases, as also observed by others **to occur very transiently at early time points, although using** total striatal membranes (Tarditi et al., 2006). A similar up-regulation of A_{2A}R was observed in the afflicted brain regions in different neurodegenerative diseases such as Alzheimer's disease (Albasanz et al., 2008; Espinosa et al., 2013), early life convulsions (Cognato et al., 2010) or attention deficit and hyper-activity disorder (Pandolfo et al., 2013), as well as upon aging (Canas et al., 2009; Rebola et al., 2003), all conditions where A_{2A}R blockade prevents spatial memory impairment (Canas et al., 2009; Cognato et al., 2010; Dall'igna et al., 2007; Prediger et al., 2005). Notably the up-regulation of A_{2A}R upon noxious brain insults is most evident in glutamatergic synapses (Cognato et al., 2010; Duarte et al., 2012; Rebola et al., 2005b), in accordance with the suggestion that A_{2A}R blockade controls learning disabilities through a normalization of aberrant plasticity in excitatory synapses (reviewed in Cunha and Agostinho, 2010). This is further supported by the lack of modification of other processes that have been also associated with early dysfunction in HD and that can be controlled by A_{2A}R; thus, our qualitative screening indicated that the genetic inactivation of A_{2A}R in R6/2 mice did not modify either the

deposition of huntingtin and the emergence of ubiquitin-containing nuclear inclusions that are hallmarks of HD (e.g. DiFiglia et al., 1997; Seo et al., 2004) and can be controlled by A_{2A}R (Chiang et al., 2009; Huang et al., 2011) or the putative astrogliosis, measured as an increased GFAP immunoreactivity that is also part of HD neuropathology (Bradford et al., 2009; Selkoe et al., 1982) and can be controlled by A_{2A}R (Matos et al., 2012; Minghetti et al., 2007). Although the observed up-regulation of presynaptic A_{2A}R, their gain of function in the control of cortico-striatal LTD and the selective amelioration of working memory deficits in R6/2 (but not WT) mice by KW6002, is suggestive of a selective ability of A_{2A}R to control the working memory deficits associated with the over-expression of mutated huntingtin, the present results do not actually allow disentangling this proposal from the equally likely possibility that the amelioration of working memory performance upon A_{2A}R deletion might instead result from an additive effect of the detrimental impact of huntingtin mutation and of the beneficial effect of A_{2A}R deletion on working memory performance also observed in control animals (Augusto et al., 2013; Wei et al., 2011; Yu et al., 2009; Zhou et al., 2009).

This selective benefit upon A_{2A}R blockade to rescue working memory deficits in prodrome and early HD should be clearly distinguished from the reported effects of A_{2A}R in the locomotor alterations that are characteristic of middle and late stages on HD. Several studies have reported dual and opposite effects of A_{2A}R on HD-associated neurochemical and motor disturbances during the evolution of HD (reviewed in Popoli et al., 2007), so that *A_{2A}R blockade* seems beneficial at *early* stages whereas it is *A_{2A}R activation* that becomes beneficial at *later* stages of the disease (Chiang et al., 2009; Chou et al., 2005; Domenici et al., 2007). Notably, this is closely correlated with electrophysiological and neurochemical evidence suggesting that the cortico-striatal glutamatergic pathway undergoes a biphasic change, from hyperactivity in the early, pre-symptomatic disease stage, to depression at late, symptomatic stages of HD models (Cepeda et al., 2007; Hong et al., 2012; Joshi et al., 2009; Klapstein et al., 2001). **This modification of the overall impact of A_{2A}R blockade during the course of the disease is also mirrored by the previously reported transient nature of the up-regulation of striatal A_{2A}R protein and mRNA levels during the**

course of the disease (Tarditi et al., 2006). This further argues for a critical role of synaptic A_{2A}R controlling cortico-striatal plasticity in the recovery of working memory selectively in the prodrome and early HD, independently of the impact of A_{2A}R on motor function or neuronal death at later stages of HD. This contention is also in agreement with the long-standing view that HD begins with a synaptic dysfunction (synaptopathy) preceding neuronal death (Deng et al., 2013; Li et al., 2003; Milnerwood and Raymond, 2010), which selectively affects corticostriatal projections (Cepeda et al., 2007; Hong et al., 2012; Joshi et al., 2009; Klapstein et al., 2001). Additionally, it should be emphasized that the presently demonstrated impact of A_{2A}R on working memory deficits are pertinent to the prodrome of HD and likely attributed to the presynaptic A_{2A}R function, and should be distinguished from the lack of direct effect of A_{2A}R alone, unless dopamine D₁ receptors are also simultaneously targeted, to control long-term reference memory deficits that emerge at later stages of HD and largely derived from the postsynaptic A_{2A}R (Tyebji et al., 2014).

The therapeutic interest of A_{2A}R antagonists to control cognitive impairments in early HD is further bolstered by our findings that the inactivation of A_{2A}R in R6/2 mice does not seem to have long-term deleterious consequences as gauged by the lack of a decrease of the survival of R6/2 mice. In fact, A_{2A}R have been proposed to be associated with the onset of motor symptoms (Dalbem et al., 2005) and with survival (Mievis et al., 2011) in HD patients and animal models. We now observed that A_{2A}R inactivation increased the survival of R6/2-CAG120 mice, while it did not significantly affect the survival of R6/2-CAG240 mice. This probably results from the ability of A_{2A}R to control seizure activity (El Yacoubi et al., 2008; El Yacoubi et al., 2009) limiting the survival of R6/2-CAG120 mice due to their mixed genetic background, a feature that is eliminated in R6/2-CAG240 mice that have a in congenic C57BL/6 background. However, this revealed therapeutic interest of A_{2A}R antagonists to manage the working memory dysfunction characteristic of the HD prodrome should be carefully balanced with the current lack of consensus about the therapeutic interest of A_{2A}R in the control of motor abnormalities characteristic of HD (Popoli et al., 2007); in fact, several studies have failed to document a motor benefit or increased survival resulting from caffeine consumption in HD patients (Simonin et al., 2013) or A_{2A}R antagonism in animal models of

HD (Mievis et al., 2011) and instead concluded on the motor benefits of A_{2A}R agonists to limit the abnormal motor phenotype of overt HD (Chou et al., 2005). This means that the window of interest to antagonize A_{2A}R to manipulate working memory deficits might be limited to the prodrome phase of HD.

In conclusion, the present study shows the ability of A_{2A}R inactivation or pharmacological blockade to control working memory deficits in the prodrome and early phases of R6/2 mice modeling HD. This was associated with an up-regulation of synaptic A_{2A}R and a gain of function of A_{2A}R to selectively control LTD in cortico-striatal pathways. Together with the lack of deleterious long-term consequences, these findings provide a proof-of-concept to propose A_{2A}R antagonists as a new therapeutic option to manage cognitive impairments and improve the quality of life of patients in the prodrome and early HD, a proposal that still needs to be validated by clinical trials that can be rapidly implemented since A_{2A}R antagonists have now been approved as anti-Parkinsonian drugs with a notable safety profile.

References

- Albasanz, J.L., Perez, S., Barrachina, M., Ferrer, I., Martín, M., 2008. Up-regulation of adenosine receptors in the frontal cortex in Alzheimer's disease. *Brain Pathol.* 18, 211-219.
- Anderson, W.W., Collingridge, G.L., 2001. The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *J. Neurosci. Methods* 108, 71-83.
- Augusto, E., Matos, M., Sévigny, J., El-Tayeb, A., Bynoe, M.S., Müller, C.E., Cunha, R.A., Chen, J.F., 2013. Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A_{2A} receptor functions. *J. Neurosci.* 33, 11390-11399.
- Bannon, N.M., Zhang, P., Ilin, V., Chistiakova, M., Volgushev, M., 2014. Modulation of synaptic transmission by adenosine in layer 2/3 of the rat visual cortex in vitro. *Neuroscience* 260, 171-184.
- Batalha, V.L., Pego, J.M., Fontinha, B.M., Costenla, A.R., Valadas, J.S., Baqi, Y., Radjainia, H., Müller, C.E., Sebastião, A.M., Lopes, L.V., 2013. Adenosine A_{2A} receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Mol. Psychiatry* 18, 320-331.
- Bradford, J., Shin, J.Y., Roberts, M., Wang, C.E., Li, X.J., Li, S., 2009. Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. *Proc. Natl. Acad. Sci. U. S. A.* 106, 22480-22485.
- Brooks, S.P., Betteridge, H., Trueman, R.C., Jones, L., Dunnett, S.B., 2006. Selective extra-dimensional set shifting deficit in a knock-in mouse model of Huntington's disease. *Brain Res. Bull.* 69, 452-457.
- Brooks, S.P., Janghra, N., Higgs, G.V., Bayram-Weston, Z., Heuer, A., Jones, L., Dunnett, S.B., 2012. Selective cognitive impairment in the YAC128 Huntington's disease mouse. *Brain Res. Bull.* 88, 121-129.
- Canas, P.M., Porciúncula, L.O., Cunha, G.M.A., Silva, C.G., Machado, N.J., Oliveira, J.M.A., Oliveira, C.R., Cunha, R.A., 2009. Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. *J. Neurosci.* 29, 14741-14751.
- Carter, R.J., Lione, L.A., Humby, T., Mangiarini, L., Mahal, A., Bates, G.P., Dunnett, S.B., Morton, A.J., 1999. Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *J. Neurosci.* 19, 3248-3257.
- Cepeda, C., Cummings, D.M., Hickey, M.A., Kleiman-Weiner, M., Chen, J.Y., Watson, J.B., Levine, M.S., 2010. Rescuing the corticostriatal synaptic disconnection in the R6/2 mouse model of Huntington's disease: exercise, adenosine receptors and ampakines. *PLoS Curr.* 2, RRN1182.
- Cepeda, C., Wu, N., André, V.M., Cummings, D.M., Levine, M.S., 2007. The corticostriatal pathway in Huntington's disease. *Prog. Neurobiol.* 81, 253-271.
- Chen, J.F., Huang, Z., Ma, J., Zhu, J., Moratalla, R., Standaert, D., Moskowitz, M.A., Fink, J.S., Schwarzschild, M.A., 1999. A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J. Neurosci.* 19, 9192-9200.
- Chiang, M.C., Chen HM, Lai HL, Chen HW, Chou SY, Chen CM, Tsai FJ, Chern Y., 2009. The A_{2A} adenosine receptor rescues the urea cycle deficiency of Huntington's disease by enhancing the activity of the ubiquitin-proteasome system. *Hum. Mol. Genet.* 18, 2929-2942.
- Chou, S.Y., Lee, Y.C., Chen, H.M., Chiang, M.C., Lai, H.L., Chang, H.H., Wu, Y.C., Sun, C.N., Chien, C.L., Lin, Y.S., Wang, S.C., Tung, Y.Y., Chang, C., Chern, Y., 2005. CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. *J. Neurochem.* 93, 310-320.
- Ciruela, F., Casadó, V., Rodrigues, R.J., Lujan, R., Burgueño, J., Canals, M., Borycz, J., Rebola, N., Goldberg, S.R., Mallol, J., Cortés, A., Canela, E.I., Lopez-Gimenez, J.F., Milligan, G., Lluís,

- C., Cunha, R.A., Ferré, S., Franco, R., 2006. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A₁-A_{2A} receptor heteromers. *J. Neurosci.* 26, 2080-2087.
- Cognato, G.P., Agostinho, P.M., Hockemeyer, J., Müller, C.E., Souza, D.O., Cunha, R.A., 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.
- Cummings, D.M., Milnerwood, A.J., Dallérac, G.M., Waights, V., Brown, J.Y., Vatsavayai, S.C., Hirst, M.C., Murphy, K.P., 2006. Aberrant cortical synaptic plasticity and dopaminergic dysfunction in a mouse model of Huntington's disease. *Hum. Mol. Genet.* 15, 2856-2868.
- Cunha, R.A., 2008. Different cellular sources and different roles of adenosine: A₁ receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A_{2A} receptor-mediated facilitation of plasticity. *Neurochem. Int.* 52, 65-72.
- Cunha, G.M.A., Canas, P.M., Melo, C.S., Hockemeyer, J., Müller, C.E., Oliveira, C.R., Cunha, R.A., 2008. Adenosine A_{2A} receptor blockade prevents memory dysfunction caused by β -amyloid peptides but not by scopolamine or MK-801. *Exp. Neurol.* 210, 776-781.
- Cunha, R.A., Agostinho, P.M., 2010. Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. *J. Alzheimers Dis.* 20 Suppl.1, S95-S116.
- d'Alcantara, P., Ledent, C., Swillens, S., Schiffmann, S.N., 2001. Inactivation of adenosine A_{2A} receptor impairs long term potentiation in the accumbens nucleus without altering basal synaptic transmission. *Neuroscience* 107, 455-464.
- Dalbem, A., Silveira, C.V., Pedroso, M.F., Breda, R.V., Werne Baes, C.V., Bartmann, A.P., da Costa, J.C., 2005. Altered distribution of striatal activity-dependent synaptic plasticity in the 3-nitropropionic acid model of Huntington's disease. *Brain Res.* 1047, 148-158.
- Dall'Igna, O.P., Fett, P., Gomes, M.W., Souza, D.O., Cunha, R.A., Lara, D.R., 2007. Caffeine and adenosine A_{2a} receptor antagonists prevent β -amyloid (25-35)-induced cognitive deficits in mice. *Exp. Neurol.* 203, 241-245.
- Dallerac, G.M., Vatsavayai, S.C., Cummings, D.M., Milnerwood, A.J., Peddie, C.J., Evans, K.A., Walters, S.W., Rezaie, P., Hirst, M.C., Murphy, K.P., 2011. Impaired long-term potentiation in the prefrontal cortex of Huntington's disease mouse models: rescue by D1 dopamine receptor activation. *Neurodegener. Dis.* 8, 230-239.
- Deng, Y.P., Wong, T., Bricker-Anthony, C., Deng, B., Reiner, A., 2013. Loss of corticostriatal and thalamostriatal synaptic terminals precedes striatal projection neuron pathology in heterozygous Q140 Huntington's disease mice. *Neurobiol. Dis.* 60, 89-107.
- DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P., Aronin, N., 1997. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 277, 1990-1993.
- Domenici, M.R., Scattoni ML, Martire A, Lastoria G, Potenza RL, Borioni A, Venerosi A, Calamandrei G, Popoli P., 2007. Behavioral and electrophysiological effects of the adenosine A_{2A} receptor antagonist SCH 58261 in R6/2 Huntington's disease mice. *Neurobiol. Dis.* 28, 197-205.
- Duarte, J.M.N., Agostinho, P.M., Carvalho, R.A., Cunha, R.A., 2012. Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcZNO10/LTJ mice. *PLoS One* 7, e21899.
- Duff, K., Paulsen, J., Mills, J., Beglinger, L.J., Moser, D.J., Smith, M.M., Langbehn, D., Stout, J., Queller, S., Harrington, D.L.; PREDICT-HD Investigators and Coordinators of the Huntington Study Group, 2010. Mild cognitive impairment in prediagnosed Huntington disease. *Neurology* 75, 500-507.
- El Yacoubi, M., Ledent, C., Parmentier, M., Costentin, J., Vaugeois, J.M., 2008. Evidence for the involvement of the adenosine A_{2A} receptor in the lowered susceptibility to pentylentetrazol-induced seizures produced in mice by long-term treatment with caffeine. *Neuropharmacology*

- El Yacoubi, M., Ledent, C., Parmentier, M., Costentin, J., Vaugeois, J.M., 2009. Adenosine A_{2A} receptor deficient mice are partially resistant to limbic seizures. *Naunyn Schmiedeberg's Arch. Pharmacol.* 380, 223-232.
- Espinosa, J., Rocha, A., Nunes, F., Costa, M.S., Schein, V., Kazlauskas, V., Kalinine, E., Souza, D.O., Cunha, R.A., Porciúncula, L.O., 2013. Caffeine consumption prevents memory impairment, neuronal damage, and adenosine A_{2A} receptors upregulation in the hippocampus of a rat model of sporadic dementia. *J. Alzheimers Dis.* 34, 509-518.
- Ferré, S., Lluís, C., Justinova, Z., Quiroz, C., Orru, M., Navarro, G., Canela, E.I., Franco, R., Goldberg, S.R., 2010. Adenosine-cannabinoid receptor interactions. Implications for striatal function. *Br. J. Pharmacol.* 160, 443-453.
- Flajolet, M., Wang, Z., Futter, M., Shen, W., Nuangchamnong, N., Bendor, J., Wallach, I., Nairn, A.C., Surmeier, D.J., Greengard, P., 2008. FGF acts as a co-transmitter through adenosine A_{2A} receptor to regulate synaptic plasticity. *Nat. Neurosci.* 11, 1402-1409.
- Gerdeman, G.L., Ronesi, J., Lovinger, D.M., 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat. Neurosci.* 5, 446-451.
- Gil, J.M., Rego, A.C., 2009. The R6 lines of transgenic mice: a model for screening new therapies for Huntington's disease. *Brain Res. Rev.* 59, 410-431.
- Giralt, A., Puigdel·l·vol, M., Carretón, O., Paoletti, P., Valero, J., Parra-Damas, A., Saura, C.A., Alberch, J., Ginés, S., 2012. Cognitive dysfunction in Huntington's disease: humans, mouse models and molecular mechanisms. *J. Huntingtons Dis.* 1, 155-173.
- Goldman-Rakic, P.S., 1996. Regional and cellular fractionation of working memory. *Proc. Natl. Acad. Sci. U. S.A.* 93, 13473-13480.
- Hockemeyer, J., Burbiel, J.C., Müller, C.E., 2004. Multigram-scale syntheses, stability, and photoreactions of A_{2A} adenosine receptor antagonists with 8-styrylxanthine structure: potential drugs for Parkinson's disease. *J. Org. Chem.* 69, 3308-3318.
- Höhn, S., Dallérac, G., Faure, A., Urbach, Y.K., Nguyen, H.P., Riess, O., von Hörsten, S., Le Blanc, P., Desvignes, N., El Massioui, N., Brown, B.L., Doyère, V., 2011. Behavioral and in vivo electrophysiological evidence for presymptomatic alteration of prefrontostriatal processing in the transgenic rat model for Huntington disease. *J. Neurosci.* 31, 8986-8997.
- Hong, S.L., Cossyleon, D., Hussain, W.A., Walker, L.J., Barton, S.J., Rebec, G.V., 2012. Dysfunctional behavioral modulation of corticostriatal communication in the R6/2 mouse model of Huntington's disease. *PLoS One* 7, e47026.
- Huang, N.K., Lin, J.H., Lin, J.T., Lin, C.I., Liu, E.M., Lin, C.J., Chen, W.P., Shen, Y.C., Chen, H.M., Chen, J.B., Lai, H.L., Yang, C.W., Chiang, M.C., Wu, Y.S., Chang, C., Chen, J.F., Fang, J.M., Lin, Y.L., Chern, Y., 2011. A new drug design targeting the adenosinergic system for Huntington's disease. *PLoS One* 6, e20934.
- Joshi, P.R., Wu, N.P., André, V.M., Cummings, D.M., Cepeda, C., Joyce, J.A., Carroll, J.B., Leavitt, B.R., Hayden, M.R., Levine, M.S., Bamford, N.S., 2009. Age-dependent alterations of corticostriatal activity in the YAC128 mouse model of Huntington disease. *J. Neurosci.* 29, 2414-2427.
- Kadowaki Horita, T., Kobayashi, M., Mori, A., Jenner, P., Kanda, T., 2013. Effects of the adenosine A_{2A} antagonist istradefylline on cognitive performance in rats with a 6-OHDA lesion in prefrontal cortex. *Psychopharmacology (Berl)*. 230, 345-352.
- Kheirbek, M.A., Britt, J.P., Beeler, J.A., Ishikawa, Y., McGehee, D.S., Zhuang, X., 2009. Adenylyl cyclase type 5 contributes to corticostriatal plasticity and striatum- dependent learning. *J. Neurosci.* 29, 12115-12124.
- Klapstein, G.J., Fisher, R.S., Zanjani, H., Cepeda, C., Jokel, E.S., Chesselet, M.F., Levine, M.S., 2001. Electrophysiological and morphological changes in striatal spiny neurons in R6/2

- Huntington's disease transgenic mice. *J. Neurophysiol.* 86, 2667-2677.
- Kung, V.W., Hassam R, Morton AJ, Jones S., 2007. Dopamine-dependent long term potentiation in the dorsal striatum is reduced in the R6/2 mouse model of Huntington's disease. *Neuroscience* 146, 1571-1580.
- Lawrence, A.D., Hodges, J.R., Rosser, A.E., Kershaw, A., French-Constant, C., Rubinsztein, D.C., Robbins, T.W., Sahakian, B.J., 1998. Evidence for specific cognitive deficits in preclinical Huntington's disease. *Brain* 121, 1329-1341.
- Li, J.Y., Plomann, M., Brundin, P., 2003. Huntington's disease: a synaptopathy? *Trends Mol. Med.* 9, 414-420.
- Lione, L.A., Carter, R.J., Hunt, M.J., Bates, G.P., Morton, A.J., Dunnett, S.B., 1999. Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. *J. Neurosci.* 19, 10428-10437.
- Lopes, L.V., Halldner, L., Rebola, N., Johansson, B., Ledent, C., Chen, J.F., Fredholm, B.B., Cunha, R.A., 2004. Binding of the prototypical adenosine A_{2A} receptor agonist CGS 21680 to the cerebral cortex of adenosine A₁ and A_{2A} receptor knockout mice. *Br. J. Pharmacol.* 141, 1006-1014.
- Lovinger, D.M., 2010. Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum. *Neuropharmacology* 58, 951-961.
- Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trotter, Y., Lehrach, H., Davies, S.W., Bates, G.P., 1996. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 87, 493-506.
- Martire, A., Tebano, M.T., Chiodi, V., Ferreira, S.G., Cunha, R.A., Köfalvi, A., Popoli, P., 2011. Pre-synaptic adenosine A_{2A} receptors control cannabinoid CB₁ receptor-mediated inhibition of striatal glutamatergic neurotransmission. *J. Neurochem.* 116, 273-280.
- Matos, M., Augusto, E., Machado, N.J., Santos-Rodrigues, A., Cunha, R.A., Agostinho, P., 2012. Astrocytic adenosine A_{2A} receptors control the amyloid- β peptide-induced decrease of glutamate uptake. *J. Alzheimers Dis.* 31, 555-567.
- Mazarakis, N.K., Cybulska-Klosowicz, A., Grote, H., Pang, T., Van Dellen, A., Kossut, M., Blakemore, C., Hannan, A.J., 2005. Deficits in experience-dependent cortical plasticity and sensory-discrimination learning in presymptomatic Huntington's disease mice. *J. Neurosci.* 25, 3059-3066.
- Menalled, L.B., Chesselet, M.F., 2002. Mouse models of Huntington's disease. *Trends Pharmacol. Sci.* 23, 32-39.
- Mievis, S., Blum, D., Ledent, C., 2011. A_{2A} receptor knockout worsens survival and motor behaviour in a transgenic mouse model of Huntington's disease. *Neurobiol. Dis.* 41, 570-576.
- Milnerwood, A.J., Cummings, D.M., Dallérac, G.M., Brown, J.Y., Vatsavayi, S.C., Hirst, M.C., Rezaie, P., Murphy, K.P., 2006. Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. *Hum. Mol. Genet.* 15, 1690-1703.
- Milnerwood, A.J., Raymond, L.A., 2010. Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. *Trends Neurosci.* 33, 513-523.
- Minghetti, L., Greco, A., Potenza, R.L., Pezzola, A., Blum, D., Bantubungi, K., Popoli, P., 2007. Effects of the adenosine A_{2A} receptor antagonist SCH 58621 on cyclooxygenase-2 expression, glial activation, and brain-derived neurotrophic factor availability in a rat model of striatal neurodegeneration. *J. Neuropathol. Exp. Neurol.* 66, 363-371.
- Nishi, A., Watanabe, Y., Higashi, H., Tanaka, M., Nairn, A.C., Greengard, P., 2005. Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1199-1204.
- Pandolfo, P., Machado, N.J., Köfalvi, A., Takahashi, R.N., Cunha, R.A., 2013. Caffeine regulates

- frontocorticostriatal dopamine transporter density and improves attention and cognitive deficits in an animal model of attention deficit hyperactivity disorder. *Eur. Neuropsychopharmacol.* 23, 317-328.
- Papp, K.V., Kaplan, R.F., Snyder, P.J., 2011. Biological markers of cognition in prodromal Huntington's disease: a review. *Brain Cogn.* 77, 280-291.
- Pekny, M., Nilsson, M., 2005. Astrocyte activation and reactive gliosis. *Glia* 50, 427-434.
- Popoli, P., Blum, D., Martire, A., Ledent, C., Ceruti, S., Abbracchio, M.P., 2007. Functions, dysfunctions and possible therapeutic relevance of adenosine A_{2A} receptors in Huntington's disease. *Prog. Neurobiol.* 81, 331-348.
- Prediger, R.D., Batista, L.C., Takahashi, R.N., 2005. Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A₁ and A_{2A} receptors. *Neurobiol. Aging*. 26, 957-964.
- Quiróz, C., Luján, R., Uchigashima, M., Simões, A.P., Lerner, T.N., Borycz, J., Kachroo, A., Canas, P.M., Orru, M., Schwarzschild, M.A., Rosin, D.L., Kreitzer, A.C., Cunha, R.A., Watanabe, M., Ferré, S., 2009. Key modulatory role of presynaptic adenosine A_{2A} receptors in cortical neurotransmission to the striatal direct pathway. *ScientificWorldJournal* 9, 1321-1344.
- Rebola, N., Sebastião, A.M., de Mendonça, A., Oliveira, C.R., Ribeiro, J.A., Cunha, R.A., 2003. Enhanced adenosine A_{2A} receptor facilitation of synaptic transmission in the hippocampus of aged rats. *J. Neurophysiol.* 90, 1295-1303.
- Rebola, N., Canas, P.M., Oliveira, C.R., Cunha, R.A., 2005a. Different synaptic and subsynaptic localization of adenosine A_{2A} receptors in the hippocampus and striatum of the rat. *Neuroscience* 132, 893-903.
- Rebola, N., Porciúncula, L.O., Lopes, L.V., Oliveira, C.R., Soares-da-Silva, P., Cunha, R.A., 2005b. Long-term effect of convulsive behavior on the density of adenosine A₁ and A_{2A} receptors in the rat cerebral cortex. *Epilepsia* 46 Suppl. 5, 159-165.
- Rebola, N., Lujan, R., Cunha, R.A., Mulle, C., 2008. Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. *Neuron* 57, 121-134.
- Rebola, N., Simões, A.P., Canas, P.M., Tomé, A.R., Andrade, G.M., Barry, C.E., Agostinho, P.M., Lynch, M.A., Cunha, R.A., 2011. Adenosine A_{2A} receptors control neuroinflammation and consequent hippocampal neuronal dysfunction. *J. Neurochem.* 117, 100-111.
- Rodrigues, R.J., Alfaro, T.M., Rebola, N., Oliveira, C.R., Cunha, R.A., 2005. Co-localization and functional interaction between adenosine A_{2A} and metabotropic group 5 receptors in glutamatergic nerve terminals of the rat striatum. *J. Neurochem.* 92, 433-441.
- Rodrigues, R.J., Canas, P.M., Lopes, L.V., Oliveira, C.R., Cunha, R.A., 2008. Modification of adenosine modulation of acetylcholine release in the hippocampus of aged rats. *Neurobiol. Aging* 29, 1597-1601.
- Rosin, D.L., Hettinger, B.D., Lee, A., Linden, J., 2003. Anatomy of adenosine A_{2A} receptors in brain: morphological substrates for integration of striatal function. *Neurology* 61, S12-S18.
- Schiffmann, S.N., Fisone, G., Moresco, R., Cunha, R.A., Ferré, S., 2007. Adenosine A_{2A} receptors and basal ganglia physiology. *Prog. Neurobiol.* 83, 277-292.
- Sebastião, A.M., Ribeiro, J.A., 2009. Triggering neurotrophic factor actions through adenosine A_{2A} receptor activation: implications for neuroprotection. *Br. J. Pharmacol.* 158, 15-22.
- Selkoe, D.J., Salazar, F.J., Abraham, C., Kosik, K.S., 1982. Huntington's disease: changes in striatal proteins reflect astrocytic gliosis. *Brain Res.* 245, 117-125.
- Seo, H., Sonntag, K.C., Isacson, O., 2004. Generalized brain and skin proteasome inhibition in Huntington's disease. *Ann. Neurol.* 56, 319-328.
- Shen, H.Y., Canas, P.M., Garcia-Sanz, P., Lan, J.Q., Boison, D., Moratalla, R., Cunha, R.A., Chen, J.F., 2013. Adenosine A_{2A} receptors in striatal glutamatergic terminals and GABAergic neurons

- oppositely modulate psychostimulant action and DARPP-32 phosphorylation. *PLoS One* 8, e80902.
- Shen, W., Flajolet, M., Greengard, P., Surmeier, D.J., 2008. Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* 321, 848-851.
- Simonin, C., Duru, C., Salleron, J., Hincker, P., Charles, P., Delval, A., Youssov, K., Burnouf, S., Azulay, J.P., Verny, C., Scherer, C., Tranchant, C., Goizet, C., Debruxelles, S., Defebvre, L., Sablonnière, B., Romon-Rousseaux, M., Buée, L., Destée, A., Godefroy, O., Dürr, A., Landwehrmeyer, B.; REGISTRY Study of the European Huntington's Disease Network, Bachoud-Levi, A.C., Richard, F., Blum, D., Krystkowiak, P.; Huntington French Speaking Network, 2013. Association between caffeine intake and age at onset in Huntington's disease. *Neurobiol. Dis.* 58: 179-182.
- Stout, J.C., Paulsen, J.S., Queller, S., Solomon, A.C., Whitlock, K.B., Campbell, J.C., Carlozzi, N., Duff, K., Beglinger, L.J., Langbehn, D.R., Johnson, S.A., Biglan, K.M., Aylward, E.H., 2011. Neurocognitive signs in prodromal Huntington disease. *Neuropsychology* 25, 1-14.
- Tarditi, A., Camurri, A., Varani, K., Borea, P.A., Woodman, B., Bates, G., Cattaneo, E., Abbracchio, M.P., 2006. Early and transient alteration of adenosine A_{2A} receptor signaling in a mouse model of Huntington disease. *Neurobiol. Dis.* 23, 44-53.
- Tebano, M.T., Martire, A., Rebola, N., Pepponi, R., Domenici, M.R., Grò, M.C., Schwarzchild, M.A., Chen, J.F., Cunha, R.A., Popoli, P., 2005. Adenosine A_{2A} 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.
- Tozzi, A., Tscherter, A., Belcastro, V., Tantucci, M., Costa, C., Picconi, B., Centonze, D., Calabresi, P., Borsini, F., 2007. Interaction of A_{2A} adenosine and D₂ dopamine receptors modulates corticostriatal glutamatergic transmission. *Neuropharmacology* 53, 783-789.
- Trueman, R.C., Brooks, S.P., Jones, L., Dunnett, S.B., 2009. Rule learning, visuospatial function and motor performance in the Hdh(Q92) knock-in mouse model of Huntington's disease. *Behav. Brain Res.* 203, 215-222.
- Tyebji, S., Saavedra, A., Canas, P.M., Pliassova, A., Delgado-García, J.M., Alberch, J., Cunha, R.A., Gruart, A., Pérez-Navarro, E., 2014. Hyperactivation of D1 and A_{2A} receptors contributes to cognitive dysfunction in Huntington's disease. *Neurobiol. Dis.* 74, 41-57.
- Van Raamsdonk, J.M., Pearson, J., Slow, E.J., Hossain, S.M., Leavitt, B.R., Hayden, M.R., 2005. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. *J. Neurosci.* 25, 4169-4180.
- Wei, C.J., Singer, P., Coelho, J., Boison, D., Feldon, J., Yee, B.K., Chen, J.F., 2011. Selective inactivation of adenosine A_{2A} receptors in striatal neurons enhances working memory and reversal learning. *Learn. Mem.* 18, 459-474.
- Wirkner, K., Gerevich, Z., Krause, T., Günther, A., Köles, L., Schneider, D., Nörenberg, W., Illes, P., 2004. Adenosine A_{2A} receptor-induced inhibition of NMDA and GABAA receptor-mediated synaptic currents in a subpopulation of rat striatal neurons. *Neuropharmacology* 46, 994-1007.
- Yu, C., Gupta, J., Chen, J.F., Yin, H.H., 2009. Genetic deletion of A_{2A} adenosine receptors in the striatum selectively impairs habit formation. *J. Neurosci.* 29, 15100-15103.
- Zhou, S.J., Zhu, M.E., Shu, D., Du, X.P., Song, X.H., Wang, X.T., Zheng, R.Y., Cai, X.H., Chen, J.F., He, J.C., 2009. Preferential enhancement of working memory in mice lacking adenosine A_{2A} receptors. *Brain Res.* 1303, 74-83.
- Zuccato, C., Valenza, M., Cattaneo, E., 2010. Molecular mechanisms and potential therapeutical targets in Huntington's disease. *Physiol. Rev.* 90, 905-981.

Figure legends

Figure 1. Genetic inactivation of A_{2A}R selectively rescues working memory deficits, independently of locomotor deficits at post-natal week 6 in the R6/2 (CAG120) mice.

Double transgenic CAG120-A_{2A}R KO and their WT littermates were generated and tested for working memory at postnatal week 6 as described in the Methods. CAG120-WT mice displayed significant deficits of working memory compared to WT-WT mice. Genetic inactivation of A_{2A}R preferentially prevented working memory error in normal as well as in CAG120 mice (**A**), without significant effect on reference memory (**B**). CAG120-WT mice at postnatal week 6 displayed locomotor deficits, compared to WT-WT mice (**C**), but the genetic inactivation of A_{2A}R did not modify the rota-rod performance in WT mice nor did it recover performance in CAG120 mice (**C**). Repeated measure ANOVA or one-way ANOVA followed by a Tukey's multiple-comparison post hoc test. * $P < 0.01$, # $P < 0.05$, $n = 12-15$ for the 8-arm maze test; $n = 13-18$ for the rotarod test.

Figure 2. Genetic inactivation of A_{2A}R prevents the impairment of working memory in R6/2 (CGA240) mice, independently of locomotor modifications.

Double transgenic CAG120-A_{2A}R KO and their WT littermates were generated and tested for working memory at postnatal month 2 and 3 as described in the Methods. At postnatal month 2, CAG240-WT mice displayed no motor deficits (**C**), but a clear deficits of working memory (**A**) and short-term reference memory (on testing days 2-3; **B**), compared to WT littermates (WT-WT). These cognitive deficits were maintained upon emergence of motor deficits at postnatal month 3 (testing days 4-5; **D**) and reference memory (testing days 3-4; **E**). Importantly, the genetic inactivation of A_{2A}R selectively reversed working and short-term reference memory deficits (comparing CAG240-A_{2A}R KO with CAG240-WT mice) both at postnatal month 2 (**A**, **B**) and postnatal month 3 (**D**, **E**), without effects on locomotion in the rota-rod test (**C**, **F**). Repeated measure ANOVA or one-way ANOVA followed by a Tukey's multiple-comparison post hoc test. * $P < 0.01$, # $P < 0.05$, $n = 12-16$.

Figure 3. Pharmacological blockade of A_{2A}R also reverses working memory deficits in R6/2 (CGA240) mice at postnatal month 3.

R6/2-CAG240 mice at postnatal month 3 were treated with the A_{2A}R antagonist KW6002 (1 mg/kg, i.p.) and tested 30 min after for their working and reference memory using the 8 arm radial maze. KW6002 significantly ameliorated working memory performance (**A**) without effects on short-term reference memory (**B**) and locomotor activity (as indicated by the entrance frequency to the arm (entrances/10 sec) (**C**) in R6/2-CAG240 mice. Repeated measure ANOVA followed by a Tukey's multiple-comparison post hoc test. *P<0.01, #P<0.05, n=7- 8.

Figure 4. Genetic inactivation of A_{2A}R does not affect ubiquitin-positive neuronal inclusion, astrogliosis and DARPP-32 phosphorylation in R6/2 mice.

Double transgenic CAG120-A_{2A}R KO, CAG240-A_{2A}R KO and their WT littermates were sacrificed for immunohistochemical analysis of ubiquitin (**A**), astrogliosis (**B**) and DARPP-32 phosphorylation at threonine-75 (DARPP-32-Thr-75, **C**), as described in the Methods. (**A**) At postnatal weeks 10, there was no qualitative difference in ubiquitin-positive neuronal intranuclear inclusions in either the cerebral cortex or striatum between CAG120-A_{2A}R KO and CAG120-WT mice. Red color-ubiquitin positive; Blue color-DAPI positive. (**B**) At postnatal day (PN) 50, R6/2-CAG120 mice displayed increased GFAP-immunoreactivity, particularly in the white matter between cortical and striatal regions and in the cortex (insert image from the box location in Fig. 4B). However, the pattern of GFAP-immunoreactivity was indistinguishable between CAG120-WT and CAG120-A_{2A}R KO mice (upper panels). Similarly, GFAP immunoreactivity was indistinguishable between CAG240-WT and CAG240-A_{2A}R KO at PN 90 (lower panels). (**C**) At PN 50, the immunoreactivity of DARPP-32-Thr-75 was markedly reduced in CAG120-A_{2A}R KO and CAG120-WT mice compared with WT-WT littermates; however, double transgenic mice (i.e. CAG120-A_{2A}R KO) did not show any further modification of DARPP-32-Thr-75-p compared to WT-A_{2A}R KO or R6/2-WT alone (40x). (**D**) The specific binding density of ³H-SCH58261 in R6/2 mice and WT littermates at PN45, as described in the Methods, showed that the binding density of ³H-SCH58261 in nerve terminal membranes of R6/2 mice was larger compared to their WT littermates

(*P<0.05, unpaired Student's *t* test, n=5).

Figure 5. Effect of A_{2A}R on LTD and LTP in cortico-striatal synapses of R6/2 mice.

Different patterns of high frequency stimulation (HFS) of the afferent cortical projections triggered LTP and LTD in the dorso-lateral striatum evaluated as changes of the amplitude of population spike (PS) electrophysiological recordings in slices collected from R6/2 mice and WT littermates at postnatal day 35, as described in the Methods. The amplitude of LTD in cortico-striatal synapses was similar ($p<0.05$) in WT and R6/2 mice (**A, B**); however, the blockade of A_{2A}R by SCH58261 (50 nM) decreased LTD amplitude only in slices from R6/2 mice (**A, D**), and was devoid of effects in WT mice (**A, C**). The amplitude of LTP was also similar between WT and R6/2 mice (**E, F**) and SCH58261 (50 nM) decreased LTP in WT (**E, G**) and R6/2 mice (**E, H**) to a similar extent (**E**). (*P<0.05, unpaired Student's *t* test, n=4-5).

Figure 6. Genetic inactivation of the A_{2A}R improved survival of CAG120 but not CAG240 mice.

Double transgenic CAG120-A_{2A}R homozygous (KO) and heterozygous (HE) and their WT littermates were generated and monitored for survival during postnatal development as described in the Methods. The survival data were analyzed by Kaplan-Meier survival curves analysis. (**A**) Genetic inactivation of A_{2A}R improved the survival time of the CAG120 line. CAG120-A_{2A}R-WT, n=8; CAG120-A_{2A}R-KO, n=7; CAG120-A_{2A}R-HE, n=10. (**B**) Survival of the CAG240 line. CAG240-A_{2A}R WT, n=7; CAG240-A_{2A}R-KO, n=8; CAG240-A_{2A}R-HE, n=9. A Log-rank (Mantel-Cox) test showed significant difference in CAG120 line ($p=0.0014$) but not in CAG240 line ($p=0.1648$).

Fig.1

Fig.2

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Figure 4

A. ubiquitin

B. GFAP

C. DARPP-32-Thr75 phosphorylation

D. A_{2A}R binding

Figure 5

Figure 6

A

B