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Caffeine and adenosine A_{2a} receptor antagonists prevent β-amyloid (25–35)-induced cognitive deficits in mice

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Abstract

Consumption of caffeine, an adenosine receptor antagonist, was found to be inversely associated with the incidence of Alzheimer's disease. Moreover, caffeine protects cultured neurons against β-amyloid-induced toxicity, an effect mimicked by adenosine A_{2A} but not A_1 receptor antagonists. We now tested if caffeine administration would prevent β-amyloid-induced cognitive impairment in mice and if this was mimicked by A_{2A} receptor blockade. One week after icv administration of the 25–35 fragment of β-amyloid (Aβ, 3 nmol), mice displayed impaired performance in both inhibitory avoidance and spontaneous alternation tests. Prolonged treatment with caffeine (1 mg/ml) had no effect alone but prevented the Aβ-induced cognitive impairment in both tasks when associated with acute caffeine (30 mg/kg) 30 min treatment before Aβ administration. The same protective effect was observed after subchronic (4 days) treatment with daily injections of either caffeine (30 mg/kg) or the selective adenosine A_{2A} receptor antagonist SCH58261 (0.5 mg/kg). This provides the first direct *in vivo* evidence that caffeine and A_{2A} receptor antagonists afford a protection against Aβ-induced amnesia, which prompts their interest for managing Alzheimer's disease.

Keywords: Adenosine; Caffeine; A_{2A} receptor; β-amyloid; Alzheimer's disease; Memory; Neuroprotection

Introduction

Caffeine, the most widely consumed psychoactive drug, acts as an adenosine receptor antagonist at non-toxic doses (Fredholm et al., 1999). Some of the most evident effects of caffeine, such as its psychomotor effects (e.g. Svenningsson et al., 1997) or its memory enhancing effects (e.g. Prediger et al., 2005), are now recognized to be due to the ability of caffeine to antagonize adenosine A_{2A} receptors. Interestingly, the blockade of A_{2A} receptors has consistently been found to afford neuroprotection against different brain insults, an effect mimicked by caffeine (reviewed in Cunha, 2005). In particular, caffeine consumption has been found to be inversely correlated with the incidence of Alzheimer's disease (AD) (Maia and de Mendonça, 2002). This is in agreement with our findings showing that caffeine and selective A_{2A} receptor antagonists afford a robust protection against β-amyloid peptide (Aβ) toxicity in cerebellar neuron cultures (Dall'Igna et al., 2003). In fact, soluble forms of Aβ are considered the most likely culprit for the early development of AD (Hardy and Selkoe, 2002). Accordingly, cerebral microinjection of Aβ causes amnesia and is considered a suitable animal model to test new protective strategies eventually relevant to manage the early phases of AD (Harkany et al., 1999). Thus, we now used an *in vivo* model of centrally administered Aβ in mice to test if acute and/or more prolonged treatment with caffeine or an A_{2A} receptor antagonist could prevent the Aβ-induced cognitive deficit in inhibitory avoidance and spontaneous alternation tasks.

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Materials and methods

Animals

Experiments were performed with male adult mice (CF1 strain) maintained in our own animal facilities under controlled environment (23±2°C, 12 h-light/dark cycle, free access to food and water) until 3–4 months old (35–45 g). All behavioral experiments were conducted between 10:00 a.m. and 2:00 p.m.

Drugs and administration procedures

The β-amyloid (25–35) peptide fragment (Aβ) was dissolved in bidistilled water at a concentration of 2 mg/ml and stored at −20°C until use. Aβ (1 mg/ml) was then incubated for 4 days at 37°C to allow the formation of birefringent fibril-like structures (Maurice et al., 1996) before intracerebroventricular (icv) administration. Mice were anesthetized with 60 mg/kg of thiopental and placed in a stereotaxic frame. A 28-gauge 5 mm-long stainless-steel needle was inserted unilaterally 1 mm lateral to bregma, 1 mm posterior and 2.5 mm deep from the pial surface (Paxinos and Franklin, 2001). Aβ or vehicle (3 μl) was delivered gradually within approximately 10 s. Correct icv administration was confirmed in preliminary experiments by injecting methylene blue into mice lateral ventricles. Control animals were injected icv with the same amount of distilled water (3 μl).

Caffeine treatment consisted of either acute, subchronic, more prolonged or combined prolonged and acute treatments. Acute treatment was a single intraperitoneal (ip) administration of caffeine at the doses of 30 or 80 mg/kg (10 ml/kg, dissolved in saline), 30 min before Aβ administration. The dose of 30 mg/kg, corresponding to the equivalent of 4–6 cups of coffee in humans, causes the maximal behavior effects in rodents (Fredholm et al., 1999), whereas the dose of 80 mg/kg is still below toxic dosage and is associated with higher antagonism of adenosine A_{2A} receptors (Quarta et al., 2004; Solinas et al., 2002). Prolonged caffeine treatment was achieved as previously described (Ciruela et al., 2006; Quarta et al., 2004) by supplying caffeine (1 mg/ml) through the drinking solution for 12 days, with Aβ administration on day 7. The combined prolonged and acute treatments consisted of 12 days of free access to the caffeine (1 mg/ml) drinking solution for 12 days and administration of caffeine (30 mg/kg) 30 min before Aβ administration. This takes advantage of the ability of prolonged caffeine treatment to desensitize A_1 receptor-mediated responses while increasing the ability of caffeine to block A_{2A} receptors (Karcz-Kubicha et al., 2003; Quarta et al., 2004). Subchronic caffeine treatment consisted in a daily administration of caffeine (30 mg/kg, ip) from 2 days before until 1 day after Aβ administration (4 days). The A_{2A} receptor antagonist SCH58261 (0.5 mg/kg) was administered subchronically as described for caffeine, i.e. it was administered ip daily from 2 days before until 1 day after Aβ administration (4 days). The dose of SCH58261 used is within the range of doses peripherally administered that have been shown to afford neuroprotection against different brain insults *in vivo* (consult Cunha, 2005).

In all experiments, we included four groups of animals: (1) control (sham-operated and water/saline treated); (2) Aβ-treated (icv injection of Aβ and water/saline treated); (3) Aβ-treated together with the different treatments with caffeine or SCH58261; (4) sham-operated and treated with caffeine or SCH58261. As the combination of prolonged and acute caffeine treatment failed to affect cognitive performance in control mice, we did not test the individual effects of only prolonged or only acute caffeine treatments in control mice. Furthermore, using the same protocol of Aβ injection, we have shown that locomotion was not altered (Dall'Igna et al., 2004), so we have not shown these control data again. Locomotion is also not expected to change due to caffeine or SCH58261 treatment as they were administered only around the time of Aβ injection, but not when behavioral tests were performed (i.e. at least 3 days after the last administration of adenosine receptor antagonists).

Inhibitory avoidance

The inhibitory avoidance is a classical test; mice are shocked when leaving a platform in a training session making them more prone to remain in the platform during a subsequent test session. We have previously used this test to monitor aversive memory in rodents (de Oliveira et al., 2005; Kazlauckas et al., 2005). Mice performance in the step-down inhibitory avoidance was examined 9 days after Aβ administration. The training apparatus is a 50×25×25 cm plastic box with a 2-cm-high, 4×6-cm-wide platform at the box center. The floor of the apparatus was made of parallel 0.1-cm caliber stainless steel bars spaced 1.0 cm apart. In the training session, the mouse was placed on the platform and latency to step-down the four paws on the grid was measured; upon stepping down, the mouse received a 2 s intermittent foot shock (three 0.5 s shocks, 0.2 mA, with a 0.25 s interval between them). Mice step-down latency was measured in a test session 24 h after training (equal to training session except for the absence of shock), keeping a ceiling time of 180 s.

Spontaneous alternation

The Y-maze is an ancillary behavioral test that allows evaluating cognitive searching behavior, although it obviously does not allow isolating memory performance (reviewed in Hughes, 2004). This test evaluates the searching behavior of animals rated as their spontaneous alternation in a Y-maze composed of 3 arms (each with 30 cm long, 20 cm height and 6 cm wide) converging to an equal angle. Eight days after distilled water or Aβ icv administration, each mouse was placed at the end of one arm and allowed to freely move through the maze during 8 min. The series of arm entries was recorded visually. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as total of alternations/(total arm entries−2), according to Maurice et al. (1996). Evaluation was performed under blind conditions to different treatments.

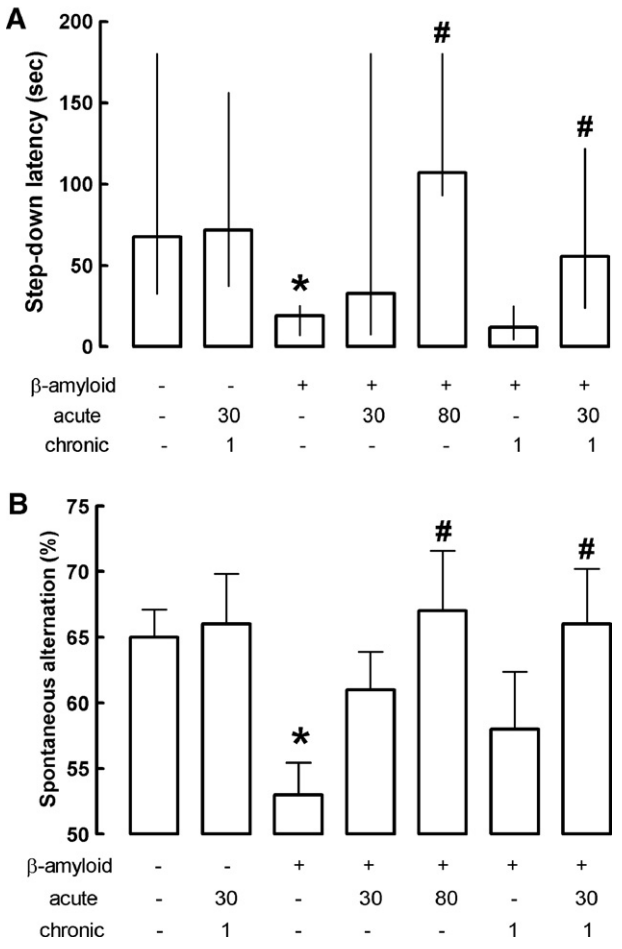

Fig. 1. Caffeine prevents β-amyloid effects on (A) inhibitory avoidance and (B) spontaneous alternation tasks. Mice were treated (3 nmol, icv) with β-amyloid (25–35) fragment (+) or vehicle (−). Caffeine acute treatment consisted of ip administration of doses of 0, 30 or 80 mg/kg, 30 min before β-amyloid icv administration. Prolonged treatment (chronic) consisted of caffeine administration in drinking water at a dose of 1 mg/ml for 6 days before until 6 days after β-amyloid administration. (A) Step-down latency is shown as median ± interquartile range and analyzed by the Kruskal–Wallis non-parametric test, followed by Mann–Whitney test. No significant modification of performance in the training trial was observed between any of the groups of mice (not shown). (B) Spontaneous alternation is expressed as mean ± SEM and analyzed by one-way ANOVA followed by Duncan post hoc test. Data are $n=25–26$ for control and amyloid-injected groups and $n=9–14$ for other groups. *$p<0.05$ vs. control (sham operated). #$p<0.05$ vs. β-amyloid.

Statistical analysis

Step-down latency is expressed as medians ± interquartile ranges since, despite being a continuous variable, values distribute in a non-normal pattern due to a fixed limit or cut-off time (ceiling time of 180 s). To determine differences among the tested groups, step-down latency values were compared using the Kruskal–Wallis non-parametric test followed by Mann–Whitney test and Bonferroni correction for α value adjustment. Spontaneous alternation values are presented as mean ± SEM and were analyzed with one-way ANOVA followed by Duncan's post hoc test. A value of $p<0.05$ was considered statistically significant.

Results

As previously reported (Maurice et al., 1996), central administration of β-amyloid (25–35) peptide (Aβ) caused an impairment of mice performance in both inhibitory avoidance (Fig. 1a) and spontaneous alternation tasks (Fig. 1b). Acute administration (ip, 30 min before Aβ administration) of caffeine at the dose of 80 mg/kg completely prevented the Aβ-induced amnesic effects in both tasks, whereas the dose of 30 mg/kg was devoid of effects (Fig. 1). Since acute administration of caffeine (up to 50 mg/kg) mostly acts by antagonism of A_1 receptors whereas higher, but still non-toxic doses (<100 mg/kg, see Fredholm et al., 1999), of caffeine applied acutely favor A_{2A} receptor antagonism (Karcz-Kubicha et al., 2003; Quarta et al., 2004; Solinas et al., 2002), these results suggest a preferential

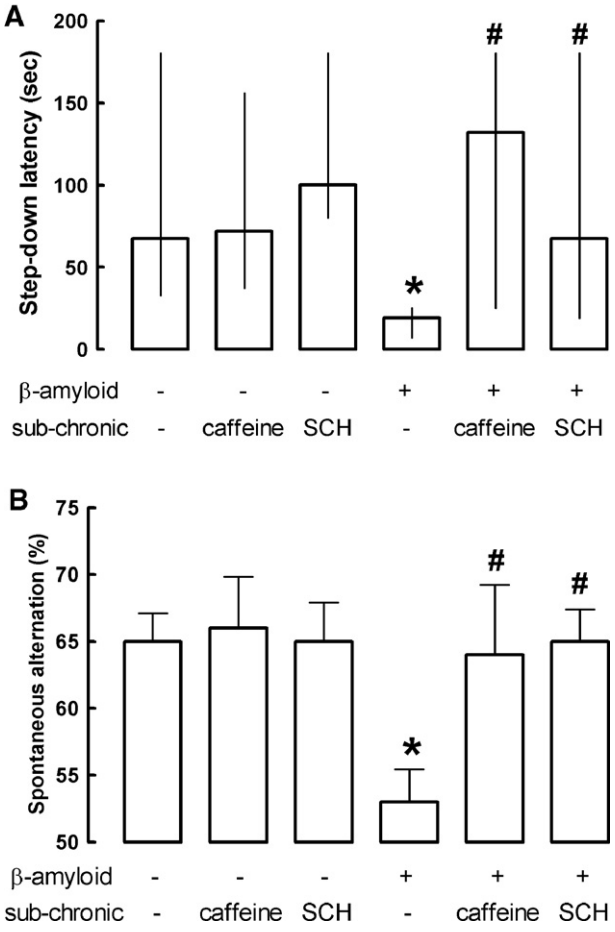

Fig. 2. Subchronic treatments with caffeine or with the selective A_{2A} receptor antagonist SCH58261 prevent β-amyloid-induced impairment of spatial memory performance. Mice were treated (3 nmol, icv) with β-amyloid (25–35) fragment (+) or vehicle (−). Subchronic treatment with caffeine (30 mg/kg) or SCH58261 (0.5 mg/kg) consisted of four daily ip injections from 2 days before until 1 day after β-amyloid administration. (A) Step-down latency is shown as median ± interquartile range and analyzed by the Kruskal–Wallis non-parametric test, followed by Mann–Whitney test. No significant modification of performance in the training trial was observed between any of the groups of mice (not shown). (B) Spontaneous alternation is expressed as mean ± SEM and analyzed by one-way ANOVA followed by Duncan post hoc test. Data are $n=25–26$ for control and amyloid-injected groups and $n=9–14$ for other groups. *$p<0.05$ vs. control (sham operated). #$p<0.05$ vs. β-amyloid.

role of A_{2A} rather than A_1 receptors in the prevention of Aβ-induced cognitive dysfunction by caffeine.

Previous studies have established that the prolonged treatment with caffeine (1 mg/ml–mean dose of 22 mg/kg/day) leads to the development of tolerance to the behavioral effects of A_1, but not A_{2A}, receptor blockade while increasing the impact of A_{2A} receptor blockade by caffeine (Karcz-Kubicha et al., 2003; Solinas et al., 2002). We found that the prolonged treatment with caffeine (1 mg/ml) was not able to prevent Aβ-induced amnesic effects, but when it was associated with the acute administration of caffeine (30 mg/kg ip 30 min before Aβ administration), the Aβ-induced amnesic effects were now abrogated (Fig. 1). This combination of prolonged (1 mg/ml) and acute (30 mg/kg, ip) caffeine treatments had no effect on the performance of control mice in both tasks (Fig. 1).

Again in an attempt to maximize the effects of caffeine on A_{2A} rather than A_1 receptors, we decided to test a subchronic treatment with a dose of caffeine of 30 mg/kg for 4 days. This subchronic administration of caffeine abolished the Aβ-induced amnesic effects (Fig. 2). To confirm the involvement of A_{2A} receptors in this protective effect of caffeine, we tested the effect of a similar subchronic administration of a selective A_{2A} receptor antagonist, SCH58261. We found that the subchronic treatment with SCH58261 (0.5 mg/kg for 4 days) completely prevented the Aβ-induced amnesic effects (Fig. 2). Subchronic treatment with SCH58261 had no effect on inhibitory avoidance or spontaneous alternation performance in control mice.

Discussion

The present results demonstrate that the blockade of adenosine A_{2A} receptors prevents β-amyloid-induced impairment of cognitive performance in the inhibitory avoidance and spontaneous alternation tasks, an effect mimicked by a subchronic administration of the non-selective adenosine receptor antagonist caffeine.

Current evidence favors the idea that soluble Aβ species play a prominent role in the pathogenesis of Alzheimer's disease (AD), a pathology that disrupts memory performance (reviewed in Hardy and Selkoe, 2002; Klein et al., 2004). One avenue of research hopefully allowing to interfere with AD is to seek for strategies to counteract Aβ-induced neuronal failure (Coleman et al., 2004; Hardy and Selkoe, 2002). This Aβ-induced cognitive disruption may primarily result from a synaptic dysfunction which then spreads to include a pattern of neuronal death (reviewed in Coleman et al., 2004), and therefore synaptically located modulatory systems may be particularly effective to counteract the initial Aβ-induced cognitive impairment. Accordingly, we now report that the blockade of adenosine A_{2A} receptors, which have a synaptic localization in the hippocampus (Rebola et al., 2005), prevents β-amyloid-induced amnesia, extending our previous observation that A_{2A} receptor antagonists prevent β-amyloid-induced toxicity in cultured neurons (Dall'Igna et al., 2003). This is in accordance with the general ability of A_{2A} receptor antagonists to afford protection against different brain insults, in particular in animal models of other neurodegenerative conditions, such as Parkinson's disease (reviewed in Cunha, 2005). Although the mechanisms by which A_{2A} receptors control the disruption of brain function in these conditions are still unresolved (reviewed in Cunha, 2005), it has been proposed that the A_{2A} receptor-mediated control of neuroinflammation could play a role (Saura et al., 2005) in accordance with the detection of A_{2A} receptors in microglia-like profiles in patients with AD (Angulo et al., 2003).

We also found that the administration of the non-selective adenosine receptor antagonist caffeine (see Fredholm et al., 1999) also prevented β-amyloid-induced impairment in spatial memory performance. This was observed upon acute administration of a dose of caffeine of 80 mg/kg, which enhances the contribution of A_{2A} receptor blockade (Quarta et al., 2004; Solinas et al., 2002) but probably lacks the selectivity for adenosine receptors since it begins affecting the activity of phosphodiesterases (see Fredholm et al., 1999). However, the neuroprotective effect was also observed by pairing prolonged caffeine treatment with an acute administration of caffeine, which causes a tolerance to the effects of A_1 receptor blockade while enhancing the behavioral effects resulting from A_{2A} receptor blockade (see Karcz-Kubicha et al., 2003; Quarta et al., 2004). Finally, the parallel protective effects recorded upon subchronic treatment with 30 mg/kg caffeine (corresponding to 4–6 cups of coffee in humans), which selectively antagonizes adenosine receptors (Fredholm et al., 1999), and with SCH58261 further strengthen the role of A_{2A} receptors in the control of Aβ-induced cognitive dysfunction. This is in agreement with our previous findings that caffeine protection against β-amyloid-induced neurotoxicity was mimicked by selective A_{2A} but not A_1 receptor antagonists (Dall'Igna et al., 2003). Interestingly, this protective effect seems not to undergo tolerance as the combination of chronic and acute caffeine treatments was more effective than each treatment alone, in accordance with the lack of desensitization over time of the neuroprotective effects of A_{2A} receptor antagonists in animal models of Parkinson's disease (Xu et al., 2002). Finally, the present observation that caffeine prevented β-amyloid-induced cognitive deficits corroborates the proposal that caffeine consumption is a protective factor in AD (Maia and de Mendonça, 2002) and bolsters the interest of A_{2A} receptor antagonists as candidate drugs to manage AD. Furthermore, both caffeine (e.g. Angelucci et al., 1999; Johnson-Kozlow et al., 2002) and selective A_{2A} receptor antagonists (Kopf et al., 1999; Prediger et al., 2005) improve mnemonic functions, an effect most evident in aged subjects (e.g. Johnson-Kozlow et al., 2002) or animals (Prediger et al., 2005) in accordance with the age-related increase in the density of A_{2A} receptors in cortical regions (e.g. Rebola et al., 2003).

In conclusion, this reported ability of caffeine and A_{2A} receptor antagonists to prevent β-amyloid-induced cognitive deficit provides the rationale to evaluate putative therapeutic actions of caffeine and of A_{2A} receptor antagonists in AD patients, with the potential to provide not only symptomatic improvement, but also a delay on cognitive decline and neurodegenerative changes.

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References

Angelucci, M.E., Vital, M.A., Cesario, C., Zadusky, C.R., Rosalen, P.L., Da Cunha, C., 1999. The effect of caffeine in animal models of learning and memory. Eur. J. Pharmacol. 373, 135–140.

Angulo, E., Casadó, V., Mallol, J., Canela, E.I., Vinals, F., Ferrer, I., Lluis, C., Franco, R., 2003. A1 adenosine receptors accumulate in neurodegenerative structures in Alzheimer disease and mediate both amyloid precursor protein processing and tau phosphorylation and translocation. Brain Pathol. 13, 440–451.

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., Lluis, C., Cunha, R.A., Ferré, S., Franco, R., 2006. Presynaptic control of striatal glutamatergic neurotransmission by adenosine A_1–A_{2A} receptor heteromers. J. Neurosci. 26, 2080–2087.

Coleman, P., Federoff, H., Kurlan, R., 2004. A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. Neurology 63, 1155–1162.

Cunha, R.A., 2005. Neuroprotection by adenosine in the brain: from A1 receptor activation to A2A receptor blockade. Purinergic Signal. 1, 111–134.

Dall'Igna, O.P., Porciúncula, L.O., Souza, D.O., Cunha, R.A., Lara, D.R., 2003. Neuroprotection by caffeine and adenosine A_{2A} receptor blockade of β-amyloid neurotoxicity. Br. J. Pharmacol. 138, 1207–1209.

Dall'Igna, O.P., Hoffmann, A., da Silva, A.L., Souza, D.O., Lara, D.R., 2004. β-amyloid treatment sensitizes mice to amphetamine-induced locomotion but reduces response to caffeine. Neurodegener. Dis. 1, 38–43.

de Oliveira, R.V., Dall'Igna, O.P., Tort, A.B., Schuh, J.F., Neto, P.F., Santos Gomes, M.W., Souza, D.O., Lara, D.R., 2005. Effect of subchronic caffeine treatment on MK-801-induced changes in locomotion, cognition and ataxia in mice. Behav. Pharmacol. 16, 79–84.

Fredholm, B.B., Battig, K., Holmen, J., Nehlig, A., Zvartau, E.E., 1999. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol. Rev. 51, 83–133.

Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353–356.

Harkany, T., Hortobagyi, T., Sasvari, M., Konya, C., Penke, B., Luiten, P.G., Nyakas, C., 1999. Neuroprotective approaches in experimental models of β-amyloid neurotoxicity: relevance to Alzheimer's disease. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 23, 963–1008.

Hughes, R.N., 2004. The value of spontaneous alteration behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci. Behav. Rev. 28, 497–505.

Johnson-Kozlow, M., Kritz-Silverstein, D., Barrett-Connor, E., Morton, D., 2002. Coffee consumption and cognitive function among older adults. Am. J. Epidemiol. 156, 842–850.

Karcz-Kubicha, M., Antoniou, K., Terasmaa, A., Quarta, D., Solinas, M., Justinova, Z., Pezzola, A., Reggio, R., Muller, C.E., Fuxe, K., Goldberg, S.R., Popoli, P., Ferré, S., 2003. Involvement of adenosine A_1 and A_{2A} receptors in the motor effects of caffeine after its acute and chronic administration. Neuropsychopharmacology 28, 1281–1291.

Kazlauckas, V., Schuh, J., Dall'Igna, O.P., Pereira, G.S., Bonan, C.D., Lara, D.R., 2005. Behavioral and cognitive profile of mice with high and low exploratory phenotypes. Behav. Brain Res. 162, 272–278.

Klein, W.L., Stine Jr., W.B., Teplow, D.B., 2004. Small assemblies of unmodified amyloid β-protein are the proximate neurotoxin in Alzheimer's disease. Neurobiol. Aging 25, 569–580.

Kopf, S.R., Melani, A., Pedata, F., Pepeu, G., 1999. Adenosine and memory storage: effects of A1 and A2 receptor antagonists. Psychopharmacology 146, 214–219.

Maia, L., de Mendonça, A., 2002. Does caffeine intake protect from Alzheimer's disease? Eur J. Neurol. 9, 377–382.

Maurice, T., Lockhart, B.P., Privat, A., 1996. Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. Brain Res. 706, 181–193.

Paxinos, G., Franklin, K.B.J., 2001. The Mouse Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, San Diego.

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 A_1 and A_{2A} receptors. Neurobiol. Aging 26, 957–964.

Quarta, D., Ferré, S., Solinas, M., You, Z.B., Hockemeyer, J., Popoli, P., Goldberg, S.R., 2004. Opposite modulatory roles for adenosine A_1 and A_{2A} receptors on glutamate and dopamine release in the shell of the nucleus accumbens. Effects of chronic caffeine exposure. J. Neurochem. 88, 1151–1158.

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., Oliveira, C.R., Cunha, R.A., 2005. Different synaptic and subsynaptic localization of adenosine A_{2A} receptors in the hippocampus and striatum of the rat. Neuroscience 132, 893–903.

Saura, J., Angulo, E., Ejarque, A., Casadó, V., Tusell, J.M., Moratalla, R., Chen, J.F., Schwarzschild, M.A., Lluis, C., Franco, R., Serratosa, J., 2005. Adenosine A_{2A} receptor stimulation potentiates nitric oxide release by activated microglia. J. Neurochem. 95, 919–929.

Solinas, M., Ferré, S., You, Z.B., Karcz-Kubicha, M., Popoli, P., Goldberg, S.R., 2002. Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. J. Neurosci. 22, 6321–6324.

Svenningsson, P., Nomikos, G.G., Fredholm, B.B., 1997. Antagonism of adenosine A2A receptors underlies the behavioural activating effect of caffeine and is associated with reduced expression of messenger RNA for NGFI-A and NGFI-B in caudate–putamen and nucleus accumbens. Neuroscience 79, 753–764.

Xu, K., Xu, Y.H., Chen, J.F., Schwarzschild, M.A., 2002. Caffeine's neuroprotection against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity shows no tolerance to chronic caffeine administration in mice. Neurosci. Lett. 322, 13–16.