Review Article

Caffeine, Adenosine Receptors, and Synaptic Plasticity

Ana Rita Costenla[a], Rodrigo A. Cunha[b] and Alexandre de Mendonça[a,c,*]
[a]*Institute of Pharmacology and Neurosciences, Faculty of Medicine and Unit of Neuroscience, Institute of Molecular Medicine, University of Lisbon, Lisbon, Portugal*
[b]*Center for Neuroscience of Coimbra, Institute of Biochemistry, Faculty of Medicine, University of Coimbra, Coimbra, Portugal*
[c]*Department of Neurology, Faculty of Medicine, University of Lisbon, Lisbon, Portugal*

Abstract. Few studies to date have looked at the effects of caffeine on synaptic plasticity, and those that did used very high concentrations of caffeine, whereas the brain concentrations attained by regular coffee consumption in humans should be in the low micromolar range, where caffeine exerts pharmacological actions mainly by antagonizing adenosine receptors. Accordingly, rats drinking caffeine (1 g/L) for 3 weeks, displayed a concentration of caffeine of *circa* 22 μM in the hippocampus. It is known that selective adenosine A_1 receptor antagonists facilitate, whereas selective adenosine A_{2A} receptor antagonists attenuate, long term potentiation (LTP) in the hippocampus. Although caffeine is a non-selective antagonist of adenosine receptors, it attenuates frequency-induced LTP in hippocampal slices in a manner similar to selective adenosine A_{2A} receptor antagonists. These effects of low micromolar concentration of caffeine (30 μM) are maintained in aged animals, which is important when a possible beneficial effect for caffeine in age-related cognitive decline is proposed. Future studies will still be required to confirm and detail the involvement of A_1 and A_{2A} receptors in the effects of caffeine on hippocampal synaptic plasticity, using both pharmacological and genetic approaches.

Keywords: A_1 receptors, A_{2A} receptors, adenosine, aging, caffeine, long-term potentiation, hippocampus, synaptic plasticity

INTRODUCTION

The cognitive enhancing properties of caffeine have long been recognized, and are experienced daily by regular coffee drinkers. Although it has been more difficult to demonstrate these cognitive enhancing effects in a formal experimental setting, caffeine improves mood and enhances psychomotor and cognitive performance in healthy volunteers, particularly on tasks measuring typing speed, simple reaction time, sustained attention, memory, and logical reasoning, as well as simulated driving [1]. Since it is generally accepted that the neurophysiological basis for learning and memory involves long-term modifications in the efficiency of synapses integrated in networks caused by specific patterns of neuronal activity [2], it would appear obvious to test whether caffeine could facilitate long-term potentiation (LTP) and related phenomena of synaptic plasticity in brain areas important for learning and memory.

WHAT IS PRESENTLY KNOWN ABOUT THE EFFECTS OF CAFFEINE ON SYNAPTIC PLASTICITY?

Rather surprisingly, few studies looked at the effects of caffeine on synaptic plasticity. In fact, re-

*Correspondence to: Alexandre de Mendonça, Laboratory of Neurosciences, Institute of Molecular Medicine, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal. Tel.: +351 217985183; Fax: +351 217999454; E-mail: mendonca@fm.ul.pt.

searchers characterizing the effects of adenosine receptors on synaptic plasticity generally used pharmacological tools that were as selective as they could get for the different adenosine receptors, and understandably neglected caffeine, which is a non-selective antagonist of adenosine receptors.

Furthermore, the few studies that looked at the effects of caffeine on synaptic plasticity used very high concentrations of caffeine, compared to the plasma levels of caffeine attained by regular consumption of coffee and other caffeine-containing beverages. One study showed that application of caffeine could induce a robust form of LTP at hippocampal Schaffer fibers/CA1 pyramid synapses *in vitro*. Caffeine was added to the recording chamber at a concentration of 10 mM [3]. Another *in vitro* study reported that caffeine could increase basal synaptic transmission and inhibit post-tetanic potentiation, but did not affect LTP, at the same synapses; the concentrations of caffeine tested in these experiments were 500 μM and 10 mM [4]. Thus, the concentrations of caffeine used in these two studies were several orders of magnitude higher than the concentration of caffeine attained in the plasma in humans by ingestion of moderate amounts of coffee, estimated to be 5–70 μM (see [5–8]). The only pharmacological action known for caffeine at the low micromolar range is the antagonism of adenosine receptors, namely adenosine A_1 and A_{2A} receptors [9,10], and possibly adenosine A_3 receptors [11]. Other effects, like inhibition of phosphodiesterases, intracellular calcium release and blockade of $GABA_A$ receptors, require higher concentrations of caffeine [10,12], although future studies still need to be carried out to either exclude or define the role of these putative targets in the central effects of caffeine. In any case, the estimation of the effects of acutely applied caffeine on LTP will first require establishing the plasma and brain concentrations of caffeine.

WHAT CONCENTRATIONS SHOULD BE TESTED TO REFLECT THE CAFFEINE LEVELS IN THE BRAIN?

We have previously found that the administration of caffeine through the drinking water at a concentration of 1 g/L affords neuroprotection and prevents memory deficits in rodents upon different noxious brain insults mimicking conditions such as diabetes, stress, early life convulsions, or Alzheimer's disease [13–15]. When allowed to have free access to caffeine in their drinking water (1 g/L) for a period of 4 weeks, rats consumed an average amount of 73 ± 3 mg caffeine/kg/day (estimation based on the average daily consumption of caffeinated water of the 6 animals during the last 2 weeks of the treatment). Upon HPLC evaluation (see e.g. [14]), these rats displayed a plasma concentration of caffeine of 32 ± 4 μM ($n = 6$), which is equivalent to the plasma concentration of caffeine found in humans consuming *circa* 6 cups of coffee daily [5,7,8]. In the same rats, the concentration of caffeine in the cerebrospinal fluid was lower than in the plasma, reaching a value of 23 ± 3 μM ($n = 6$). Finally, 0.409 ± 0.011 μg of caffeine were recovered in the hippocampal extracts ($n = 6$); this corresponds to an estimated concentration of 22 μM of caffeine, assuming a total hippocampal volume of 95 μL [16,17]. Since the brain concentration of caffeine is within 80% of its plasma concentration, we would recommend a caffeine concentration of about 30 μM to probe the effects of caffeine on synaptic plasticity in hippocampal slices.

EFFECTS OF ADENOSINE A_1 RECEPTOR ANTAGONISTS AND ADENOSINE A_{2A} RECEPTOR ANTAGONISTS ON SYNAPTIC PLASTICITY

Since, as mentioned above, caffeine is a non-selective adenosine A_1 and A_{2A} receptor antagonist, the putative effects of relevant concentrations of caffeine on synaptic plasticity could to a certain extent be inferred from the known effects of selective adenosine A_1 receptor antagonists and selective adenosine A_{2A} receptor antagonists on synaptic plasticity. The modulation exerted by adenosine receptors on synaptic plasticity was previously reviewed [18], but a summary of the key finding will be presented.

Usually compounds that are agonists of adenosine A_1 receptors, like adenosine itself, attenuate LTP, whereas selective antagonists of A_1 receptors, like 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), facilitate LTP [18]. This facilitatory effect of A_1 receptor antagonists is more evident when using relatively weak LTP induction protocols, namely theta burst stimulation, and may be more difficult to detect when using strong stimulation protocols, namely high frequency stimulation, closer to LTP saturation conditions. For example, a theta-burst stimulation protocol (only 3 trains of 3 stimuli each) could not elicit LTP under control conditions in one pathway at Schaffer fibers/CA1 pyramid synapses *in vitro*, but was able to induce robust LTP either in

the same pathway, or in the other convergent pathway, after adding DPCPX (50 nM) to the superfusion medium [19]. The same selective adenosine receptor antagonist could restore the LTP decay observed in Schaffer fibers/CA1 basal dendrite synapses in middle-aged animals [20]. This contrasts with our recent observations that DPCPX has discrete effects on LTP amplitude in middle aged rats and is virtually devoid of effects on LTP amplitude in hippocampal slices from aged rats [21].

The effects of compounds that act upon adenosine A_{2A} receptors on phenomena of synaptic plasticity have been initially somewhat inconsistent, possibly because of the moderate selectivity of the antagonists available at that time [18]. The A_{2A} receptor agonist, CGS 21680, facilitated LTP at Schaffer fibers/CA1 pyramid synapses in hippocampal slices [22,23]. When tested at these same synapses, the highly selective A_{2A} receptor antagonist, SCH 58261 (50 nM), attenuated LTP *in vitro* [21] and *in vivo* [24], an effect which was more prominent in aged animals [21]. The same A_{2A} receptor antagonist also blocked LTP of N-methyl-D-aspartate (NMDA) mediated responses at mossy fibers/CA3 pyramid synapses *in vitro* [25]. These more recent data, taken together, suggest an important role for A_{2A} receptors in controlling LTP at distinct hippocampal synapses.

Regarding adenosine A_3 receptors, their level of expression in the brain is low [26], albeit they are located in hippocampal nerve terminals [27]. Nevertheless, one study showed that an adenosine A_3 receptor agonist, Cl-IBMECA (100 nM), could increase LTP at Schaffer fibers/CA1 pyramid synapses *in vitro*, an effect prevented by a selective A_3 receptor antagonist, MRS 1191 (10 μM). The antagonist was itself devoid of effect, suggesting that endogenous adenosine does not tonically modulate LTP in the hippocampus by activating A_3 receptors [28].

In conclusion, selective adenosine A_1 receptor antagonists facilitate, whereas selective adenosine A_{2A} receptor antagonists attenuate, LTP in the hippocampus. Pharmacological studies have shown that caffeine is a low affinity antagonist of both A_1 and A_{2A} receptors, with slightly higher affinity for A_{2A} receptors in native brain preparations (e.g. [29]). Based on these pharmacological properties of caffeine, it would be difficult to predict what could be its action on LTP at concentrations comparable to those attained by regular consumption in humans.

Preliminary evidence from our laboratories suggests that caffeine attenuates frequency-induced LTP in hippocampal slices in a manner similar to selective adenosine A_{2A} receptor antagonists. Electrophysiological recordings of field excitatory postsynaptic potentials (fEPSP) were obtained from hippocampal slices taken from male Wistar rats as previously described [19]. Two separate sets of the Schaffer fibers/CA1 pyramid synapses (S1 and S2) were alternately stimulated at a basal frequency of 0.05 Hz. LTP was induced by high-frequency stimulation pattern (HFS, 1 train of 100 Hz, 100 stimuli) in one pathway (control) under control conditions, and afterwards in the other pathway (test) in the presence of caffeine (30 μM). Caffeine was added at least 45 minutes before applying the HFS train to the test pathway and was present throughout the rest of the experiment. As depicted in Fig. 1A, when a HFS train was delivered to hippocampal slices taken from young adult rats (10–15 weeks), there was a sustained increase of synaptic efficiency, i.e., there was a LTP with a magnitude of 97.0 $\pm$ 3.8% over baseline (LTP quantified as the % increase in the average value of the fEPSP slope taken from 50 to 60 min after HFS in relation to the average value of the fEPSP slope during the 10 min that preceded HFS; $n = 5$; Fig. 1A). Caffeine (30 μM) decreased the magnitude of HFS-induced LTP to 53.4 $\pm$ 7.3% over baseline ($n = 5$, $P < 0.05$; paired two-tailed Student's t test; comparing the LTP values in the absence and in the presence of caffeine; Fig. 1A).

Although the analogy of the inhibitory effects of caffeine on high frequency-induced LTP with the known inhibitory effects of selective adenosine A_{2A} receptor antagonists is striking, it should be experimentally confirmed in the future. The prediction would be that the inhibitory effects of caffeine on LTP would be occluded when a selective adenosine A_{2A} receptor antagonist is applied throughout all the experiment.

ROLE OF ADENOSINE A_1 AND A_{2A} RECEPTORS IN YOUNG AND AGED ANIMALS

Similar experiments were performed in slices derived from aged rats (70–80 weeks old). HFS stimulation also triggered LTP, which reached a magnitude of 99.8 $\pm$ 7.3% over baseline in control conditions ($n = 4$; Fig. 1B). As occurred in young adult rats, caffeine (30 μM) decreased the magnitude of HFS-induced LTP to 36.3 $\pm$ 8.2% of baseline ($n = 4$, $P < 0.05$; paired two-tailed Student's t test; Fig. 1B). These preliminary results thus suggest that the inhibitory effect of caffeine on the control of LTP magnitude is maintained both in young adult and in aged animals. Again it is hy-
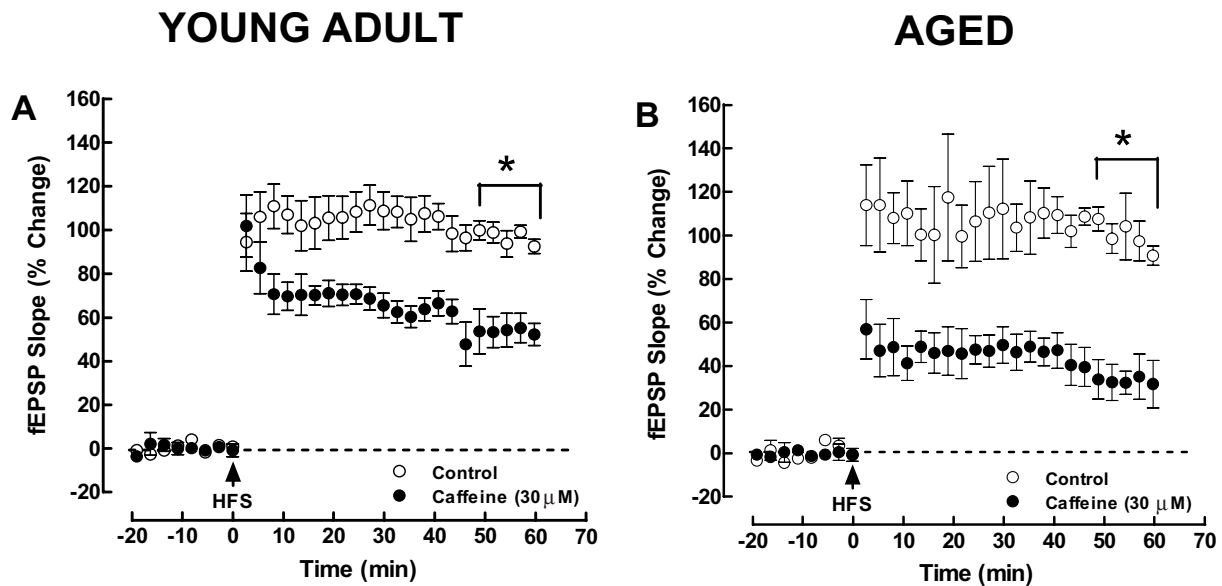
YOUNG ADULT

AGED

Fig. 1. Caffeine inhibits long-term potentiation (LTP). Caffeine (30 μM, filled symbols) decreased the magnitude of high frequency stimulation (HFS)-induced long-term potentiation (LTP) of the field excitatory postsynaptic potentials (fEPSP) in the Schaffer pathway in hippocampal slices from both young adult (A) and aged rats (B). Data are mean $\pm$ 1SEM of 4–5 experiments carried out in slices from different rats. *$P < 0.05$, LTP significantly different between control (absence of added drugs; open symbols) and the presence of 30 μM caffeine (filled symbols), Student's paired t test.

pothesised that A_{2A} receptors may be the main target of caffeine to control of LTP in aged rats since there is an age-associated increase of A_{2A} receptor density and function, associated with the decreased A_1 receptor density and function [21,30–33]. Thus, the effects of caffeine on LTP are preserved upon aging, a conclusion of main importance for the interpretation of the beneficial effects of caffeine in neurodegenerative conditions that are prevalent in the elderly.

DIFFERENTIAL ROLE OF ADENOSINE A_1 AND A_{2A} RECEPTORS UNDER CONDITIONS OF BASAL FREQUENCY STIMULATION AND HIGH FREQUENCY STIMULATION

Under conditions of high frequency-induced synaptic plasticity, endogenous extracellular adenosine mainly activates facilitatory A_{2A} receptors; in contrast, under conditions of basal frequency synaptic transmission, endogenous extracellular adenosine predominantly activates inhibitory A_1 receptors (reviewed in [34]). Since the effects of caffeine could be largely directed by the physiological conditions that determine whether the predominant signaling is dominated by A_1 receptors or by A_{2A} receptors (both of which can be antagonized nearly equi-efficiently by caffeine), understand-

ing the different (and complementary) roles of A_1 and A_{2A} receptors in the control of basal frequency synaptic transmission and synaptic plasticity is required.

A series of different approaches have allowed devising a scenario supporting a differential role for A_1 and A_{2A} receptors in the control of basal frequency synaptic transmission and plasticity, which is essentially based on the different sources of adenosine responsible for the activation of both receptors together with a crosstalk between A_1 and A_{2A} receptors (reviewed in [34]). Thus, initial studies combining biochemical, neurochemical and electrophysiological studies have supported the idea that the activation of A_{2A} receptors seems to require a particular source of adenosine, which is formed from the action of synaptically-located ecto-nucleotidases [35] upon extracellular catabolism of synaptically-released ATP [25,36]. Interestingly, the release of ATP from activated nerve terminals occurs in small amounts at low frequencies of stimulation but occurs in disproportionally larger amounts upon high frequency stimulation of nerve terminals [37–39]. This provides a neurochemical basis for the selective engagement of A_{2A} receptors at high rather than low frequencies of nerve stimulation [34], since only during the former, but not the latter, conditions is there a localised synaptic formation of ATP-derived adenosine able to activate the A_{2A} receptors that are highly en-

riched in synapses in the hippocampus [40]. In contrast, A_1 receptors are effectively activated by extracellular adenosine, which is either released as such or formed from ATP released from astrocytes [41] in a manner dependent on global nerve activity [42]. Thus, under basal frequency stimulation, there is an increase of extracellular adenosine levels [42] and an on-going inhibitory tonus provided by activation of inhibitory A_1 receptors [43]. This A_1 receptor system is rather effective and able to completely block glutamatergic synaptic transmission in the hippocampus if maximally activated (see e.g. [44]). This poses a problem to understand the possibility of increasing the efficiency of hippocampal synapses, for instance upon high frequency stimulation (LTP). In other words, increased frequency of stimulation enhances the production of extracellular adenosine [42], which would be expected to fully block synaptic transmission through A_1 receptor activation. Therefore, the expression of enhanced synaptic transmission upon high frequency stimulation should require switching off A_1 receptors. Interestingly, we have found that the activation of A_{2A} receptors is able to decrease functioning of A_1 receptors through alternative (or combined) mechanisms involving a protein kinase C-mediated desensitization of A_1 receptors [45, 46] and a heteromerization of A_1 and A_{2A} receptors whereby the activation of A_{2A} receptors switches off A_1 receptors [29]. Merging these two lines of evidences presented above, one ends up with a likely scenario able to explain the predominant role of A_1 receptors under basal frequency stimulation and a predominant role of A_{2A} receptors in synapses undergoing plasticity changes upon high frequency stimulation (reviewed in [34]).

Adenosine A_1 receptors are constitutively activated in all glutamatergic synapses and act as a general threshold barrier that tonically restraints synaptic transmission and, to a lower extent, LTP (reviewed in [34]). In particular at synapses undergoing plastic changes, there is a large ATP release and the resulting locally formed adenosine selectively engages A_{2A} receptors only in these 'activated' synapses to switch off A_1 receptors and assist implementation of LTP through potentiation of NMDA-mediated responses [25]. In parallel, through heterosynaptic depression [47] involving the activation of the astrocytic syncytium surrounding the 'activated' synapse [48], more adenosine is generated in neighbouring 'non-activated' synapses which are expected to suffer a greater A_1 receptor-mediated inhibition [49]. Thus, the combined engagement of A_{2A} receptors in the activated synapses with the glob-

al increased activation of A_1 receptors in all synapses (except the activated synapses, where A_1 receptors are switched off) contributes to define salience in complex neuronal circuits where only one group of synapses is activated to encode relevant information (reviewed in [34]).

Following the previous discussion, we might expect that caffeine, beyond antagonizing adenosine A_{2A} receptors to modulate high frequency-induced synaptic plasticity, would also antagonize adenosine A_1 receptors to control basal frequency synaptic transmission. Although this has not yet been confirmed experimentally, the available evidence is compatible with this hypothesis. Thus, caffeine, at low micromolar concentrations, is known to increase basal frequency synaptic transmission in the Schaffer fibers likely through antagonism of A_1 receptors [50]. Also in experiments from our laboratories, when applied acutely to hippocampal slices obtained from young adult rats (10–15 weeks), stimulated at a basal frequency of 0.05 Hz, caffeine (30 μM) caused a facilitation of synaptic transmission that reached an amplitude of $34.8 \pm 5.9\%$ of baseline (facilitation quantified as the % increase in the average value of the fEPSP slope taken from 20 to 35 min after beginning application of caffeine in relation to the average value of the fEPSP slope during the 10 min that preceded application of caffeine; $n = 5$, $P < 0.05$; paired two-tailed Student's t test; Fig. 2A). In a similar way, in slices derived from aged animals (70–80 weeks old), caffeine (30 μM) also enhanced basal frequency synaptic transmission, a facilitation that reached an amplitude of $55.7 \pm 8.0\%$ of baseline ($n = 5$; $P < 0.05$; paired two-tailed Student's t test; Fig. 2B). This facilitatory effect of caffeine on basal synaptic transmission is qualitatively similar to that obtained when applying a selective A_1 receptor antagonist or upon removal of endogenous extracellular adenosine (using adenosine deaminase) in either young adult or aged rats [31].

POSSIBLE RELEVANCE OF THE EFFECTS OF CAFFEINE ON LTP FOR MEMORY PERFORMANCE

Apart from its intrinsic scientific interest, the determination of the effects of caffeine on synaptic transmission and plasticity are largely driven by the striking ability of chronic caffeine consumption to prevent memory dysfunction in several animal models of disease [51,52]. This also seems to occur in humans, where it is observed that caffeine consumption
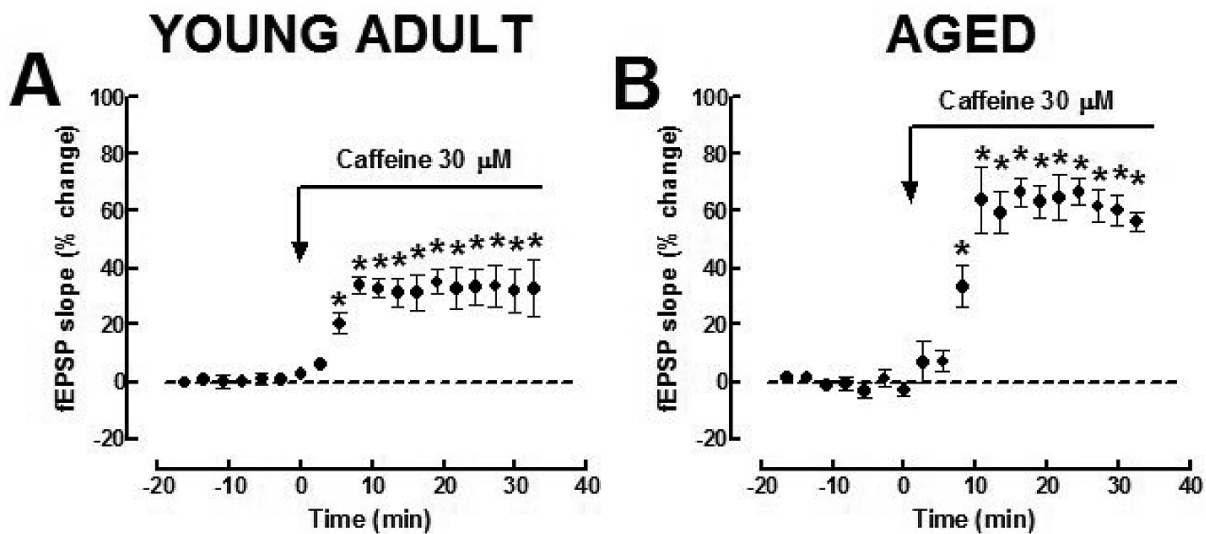
Fig. 2. Caffeine facilitates basal synaptic transmission. A) Caffeine (30 μM) enhanced the amplitude of the field excitatory postsynaptic potentials (fEPSP) in the Schaffer pathway upon basal stimulation (0.05 Hz) of hippocampal slices both from young adult (A) and from aged rats (B) in a manner similar to that caused by administration of selective A_1 receptor antagonists (data not shown). Data are mean $\pm$ 1SEM of 5 experiments carried out in slices from different rats. *$P < 0.05$, comparing the mean fEPSP slopes at different time points with the average value of the fEPSP slope during the 10 min that preceded application of caffeine, $n = 5$, Student's two-tailed paired t test, no correction made for multiple comparisons.

may prevent age-related cognitive impairment [53] (but see [54]) and may be inversely associated with the incidence of Alzheimer's disease [55,56]. Interestingly, animal studies have largely concluded that the benefits afforded by chronic caffeine consumption in terms of attenuating memory impairment upon several noxious stimuli [51,52] are mainly mimicked by selective antagonists of A_{2A} rather than A_1 receptors. Given that LTP has been conceived as a possible neurophysiological correlate of learning and memory (see [2,57]), the present observation that the effect of caffeine on LTP is qualitatively similar to the effects of selective A_{2A} receptor antagonists bolsters the hypothesis that this effect of caffeine on LTP may be related to the ability of caffeine to attenuate the impairment of memory performance. This parallel between the memory-restoring properties of caffeine (possibly through A_{2A} receptor antagonism) and the presently observed inhibition of LTP by caffeine (also possibly through A_{2A} receptor antagonism) makes it important to discuss a general incorrect assumption about a parallel relationship between the magnitude of LTP and memory performance. This widespread idea probably arose from the pioneering observations that manipulations blocking LTP also severely reduced performance in learning and memory-related tasks (reviewed in [2]). However, this sustains, at best, that LTP is required for memory processing but it should not be taken as evidence that

LTP and memory are linearly related. In fact, it has previously been elegantly discussed that correct processing of memory-related information should be dependent on defining the salience of information to be encoded, a process that requires not only potentiation of synaptic strength but also depression and depotentiation (e.g. [2,58,59]), both homosynaptic and heterosynaptic (e.g. [60,61]). For that reason, there are several previous manipulations of different proteins that resulted in an enhanced LTP coupled to decreased performance in memory-related tasks (e.g. [62–66]). Thus, it is understandable that caffeine (possibly through A_{2A} receptor antagonism) causes a decrease of LTP magnitude while restoring memory dysfunction, if one considers that caffeine and A_{2A} receptor antagonists act as normalizers rather than facilitators of synaptic plasticity and memory (discussed in [34]; see also Cunha and Agostinho, this issue).

This discussion should not exclude the possibility that the effects of caffeine on memory performance might target brain structures apart from the hippocampus and involve the control of processes that indirectly regulate memory performance. For instance, caffeine improves sustained attention [1], and attention effectively modulates the encoding and long-term retrieval of memories (e.g. [67,68]). However, since it has been shown that caffeine can have additional effects on memory performance apart from its effects on

attention, motivation, vigilance and alertness (e.g. [69, 70]), it is expected that the effect of caffeine on memory might also result from direct effects of caffeine on brain circuits directly related with memory processing amongst which stem hippocampal circuits.

SOME CONSIDERATIONS FOR FUTURE STUDIES OF CAFFEINE ON SYNAPTIC PLASTICITY

It should be noted that, so far, it was observed that caffeine attenuates frequency-induced LTP in the Schaffer pathway, but it would be interesting to test if similar effects of caffeine are also present in different excitatory synapses, particularly at the mossy fibers/CA3 pyramid synapses, where a selective adenosine A_{2A} receptor antagonist could block LTP of NMDA receptor-mediated responses [25].

Although we report that caffeine controlled LTP amplitude when induced by high frequency stimulation, there are several patterns of frequency-induced synaptic plasticity (e.g. theta-burst stimulation) where possible effects of caffeine remain to be determined. In this respect, it should be emphasized that the activation of adenosine A_{2A} receptors is not expected to be a mandatory process to implement LTP, unlike the recruitment of NMDA receptors. Instead, A_{2A} receptors are expected to assist (or facilitate) the implementation of LTP. And it still remains to be explored under which conditions of plasticity A_{2A} receptors might play determinant or passive roles. It must also be taken into account that caffeine is expected to cause a partial blockade of A_{2A} receptors, in contrast to selective A_{2A} receptor antagonists, which were used at a supramaximal (but selective) concentration to nearly fully block A_{2A} receptors. For instance, the engagement of A_{2A} receptors at the neuromuscular junction was found to be possible only with intermittent burst of high frequency stimulation rather than with a single continuous train of high frequency stimulation [71], which might be related to a different pattern of ATP release with distinct types of synaptic plasticity induction protocols that may determine the recruitment of A_{2A} receptors.

In the *in vitro* experiments on synaptic plasticity described, caffeine is usually applied in the bath superfusing the slices. It would be important to know how a prolonged and intermittent ingestion of caffeine in the experimental animals, mimicking the caffeine consumption pattern in humans, would affect synaptic plasticity elicited in slices taken from these animals. *In vivo* experiments in animals with chronically implanted electrodes would be particularly relevant for that purpose.

Another interesting point is that we have determined the effects of caffeine in male rats but it is not known what might be the effect of caffeine in female rats. This question is particularly important since there are numerous reports of sex differences in the ability of caffeine to afford neuroprotection. For instance, there is an inverse association between caffeine intake and the incidence of Parkinson's disease, which is more evident in men than in women [72], possibly due to the interfering effect of estrogen replacement therapies [73], as also suggested in animal studies [74]. In contrast, the age-related cognitive decline was attenuated by caffeine consumption in women in one study [53], whereas another study reported an inverse association between caffeine intake and the risk of dementia that was stronger in men [56]. Future work should explore whether there are differences between the effects of caffeine both on learning and memory performance and on synaptic plasticity between males and females, to help clarify this sex difference issue.

Finally, it would be of interest to test if caffeine selectively modulates long term depression and depotentiation as well as other types of shorter or longer plasticity phenomena, and to explore the mechanisms linking the control by caffeine of synaptic plasticity and its ability to modulate learning and memory performance.

CONCLUSIONS

Concentrations of caffeine above $50\,\mu M$, used in previous studies describing the effects of caffeine on LTP, are probably not representative of the brain concentrations of caffeine obtained by ingestion of moderate amounts of coffee and caffeine-containing beverages in humans. The present study provides the first description that relevant concentrations of caffeine inhibit the magnitude of LTP triggered by high-frequency stimulation (possibly via A_{2A} receptors), in contrast to the excitatory effects of caffeine on basal synaptic transmission (possibly via A_1 receptor antagonism). These effects of caffeine appear to be maintained in aged animals, possibly as a result of balanced change of the density and efficiency of A_1 and A_{2A} receptors [30–33] and of the sources of endogenous extracellular adenosine [75]. This observation is of particular importance when considering the possible beneficial effect for caffeine in age-related cognitive decline. Future studies will still be required to confirm and detail the involve-

ment of A_1 and A_{2A} receptors in the effects of caffeine on hippocampal synaptic plasticity, using both pharmacological and genetic approaches.

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REFERENCES

[1] Rogers PJ, Dernoncourt C (1998) Regular caffeine consumption: a balance of adverse and beneficial effects for mood and psychomotor performance. *Pharmacol Biochem Behav* **59**, 1039-1045.

[2] Martin SJ, Grimwood PD, Morris RGM (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. *Ann Rev Neurosci* **23**, 649-711.

[3] Martín ED, Buño W (2003) Caffeine-mediated presynaptic long-term potentiation in hippocampal CA1 pyramidal neurons. *J Neurophysiol* **89**, 3029-3038.

[4] Lee W-L, Anwyl R, Rowan M (1987) Caffeine inhibits post-tetanic potentiation but does not alter long-term potentiation in the rat hippocampal slice. *Brain Res* **426**, 250-256.

[5] Lelo A, Birkett DJ, Robson RA, Miners JO (1986) Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. *Br J Clin Pharmacol* **22**, 177-182.

[6] Kaplan GB, Greenblatt DJ, Leduc BW, Thompson ML, Shader RI (1989) Relationship of plasma and brain concentrations of caffeine and metabolites to benzodiazepine receptor binding and locomotor activity. *J Pharmacol Exp Ther* **248**, 1078-1083.

[7] Denaro CP, Brown CR, Wilson M, Jacob P 3rd, Benowitz NL (1990) Dose-dependency of caffeine metabolism with repeated dosing. *Clin Pharmacol Ther* **48**, 277-285.

[8] Benowitz NL, Jacob P 3rd, Mayan H, Denaro C (1995) Sympathomimetic effects of paraxanthine and caffeine in humans. *Clin Pharmacol Ther* **58**, 684-691.

[9] Daly JW, Butts-Lamb P, Padgett W (1983) Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell Mol Neurobiol* **3**, 69-80.

[10] Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* **51**, 83-133.

[11] Björklund O, Halldner-Henriksson L, Yang J, Eriksson TM, Jacobson MA, Daré E, Fredholm BB (2008) Decreased behavioral activation following caffeine, amphetamine and darkness in A_3 adenosine receptor knock-out mice. *Physiol Behav* **95**, 668-676.

[12] Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Rev* **17**, 139-170.

[13] Cunha RA, Cognato GP, Kaster MP, Duarte JMN, Canas PM (2008) Chronic caffeine consumption prevents memory deficits in animal models of diabetic neuropathy, chronic unpredictable stress or juvenile convulsions. *Soc Neurosci Abstr*, Program No. 291.2.

[14] Duarte JMN, Carvalho RA, Cunha RA, Gruetter R (2009) Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. *J Neurochem* **111**, 368-379.

[15] Cognato GP, Agostinho PM, Hockemeyer J, Müller CE, Souza DO, Cunha RA (2010) Caffeine and adenosine A_{2A} receptor antagonists prevent memory impairment and synaptotoxicity in adult rats triggered by a convulsive episode in early life. *J Neurochem* **112**, 453-462.

[16] Wolf OT, Dyakin V, Vadasz C, de Leon MJ, McEwen BS, Bulloch K (2002) Volumetric measurement of the hippocampus, the anterior cingulate cortex, and the retrosplenial granular cortex of the rat using structural MRI. *Brain Res Protoc* **10**, 41-46.

[17] Wolf OT, Dyakin V, Patel A, Vadasz C, de Leon MJ, McEwen BS, Bulloch K (2002) Volumetric structural magnetic resonance imaging (MRI) of the rat hippocampus following kainic acid (KA) treatment. *Brain Res* **934**, 87-96.

[18] de Mendonça A, Ribeiro JA (2001) Adenosine and synaptic plasticity. *Drug Dev Res* **52**, 283-290.

[19] Costenla AR, de Mendonça A, Ribeiro JA (1999) Adenosine modulates synaptic plasticity in hippocampal slices from aged rats. *Brain Res* **851**, 228-234.

[20] Rex CS, Kramár EA, Colgin LL, Lin B, Gall CM, Lynch G (2005) Long-term potentiation is impaired in middle-aged rats: regional specificity and reversal by adenosine receptor antagonists. *J Neurosci* **25**, 5956-5966.

[21] Costenla AR, Diógenes MJ, Ribeiro JA, Cunha RA, de Mendonça A (2008) Enhanced role of adenosine A_{2A} receptors on long-term potentiation in the rat hippocampus upon aging. *Soc Neurosci Abstr*, Program No. 541.18.

[22] de Mendonça A, Ribeiro JA (1994) Endogenous adenosine modulates long-term potentiation in the hippocampus. *Neuroscience* **62**, 385-390.

[23] Almeida T, Rodrigues RJ, de Mendonça A, Ribeiro JA, Cunha RA (2003) Purinergic P_2 receptors trigger adenosine release leading to adenosine A_{2A} receptor activation and facilitation of long-term potentiation in rat hippocampal slices. *Neuroscience* **122**, 111-121.

[24] Fontinha BM, Delgado-García JM, Madroñal N, Ribeiro JA, Sebastião AM, Gruartet A (2009) Adenosine A_{2A} receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice. *Neuropsychopharmacology* **34**, 1865-1874.

[25] Rebola N, Lujan R, Cunha RA, 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.

[26] Rivkees SA, Thevananther S, Hao H (2000) Are A_3 adenosine receptors expressed in the brain? *Neuroreport* **11**, 1025-1030.

[27] Lopes LV, Rebola N, Costenla AR, Halldner L, Jacobson MA, Oliveira CR, Richardson PJ, Fredholm BB, Ribeiro JA, Cunha RA (2003) Adenosine A$_3$ receptors in the rat hippocampus: lack of interaction with A$_1$ receptors. *Drug Dev Res* **58**, 428-438.

[28] Costenla AR, Lopes LV, de Mendonça A, Ribeiro JA (2001) A functional role for adenosine A$_3$ receptors: modulation of synaptic plasticity in the rat hippocampus. *Neurosci Lett* **302**, 53-57.

[29] Ciruela F, Casadó V, Rodrigues RJ, Luján R, Burgueño J, Canals M, Borycz J, Rebola N, Goldberg SR, Mallol J, Cortés A, Canela EI, Lopez-Gimenez JF, Milligan G, Lluis C, Cunha RA, Ferré S, Franco R (2006) Presynaptic control of striatal glutamatergic neurotransmission by adenosine A$_1$-A$_{2A}$ receptor heteromers. *J Neurosci* **26**, 2080-2087.

[30] Cunha RA, Constantino MC, Sebastião AM, Ribeiro JA (1995) Modification of A$_1$ and A$_{2a}$ adenosine receptor binding in aged striatum, hippocampus and cortex of the rat. *Neuroreport* **6**, 1583-1588.

[31] Sebastião AM, Cunha RA, de Mendonça A, Ribeiro JA (2000) Modification of adenosine modulation of synaptic transmission in the hippocampus of aged rats. *Br J Pharmacol* **131**, 1629-1634.

[32] Rebola N, Sebastião AM, de Mendonça A, Oliveira CR, Ribeiro JA, Cunha RA (2003) Enhanced adenosine A$_{2A}$ receptor facilitation of synaptic transmission in the hippocampus of aged rats. *J Neurophysiol* **90**, 1295-1303.

[33] Canas PM, Duarte JMN, Rodrigues RJ, Köfalvi A, Cunha RA (2009) Modification upon aging of the density of presynaptic modulation systems in the hippocampus. *Neurobiol Aging* **30**, 1877-1884.

[34] Cunha RA (2008) Different cellular sources and different roles of adenosine: A$_1$ receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A$_{2A}$ receptor-mediated facilitation of plasticity. *Neurochem Int* **52**, 65-72.

[35] Cunha RA (2001) Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. *Neurochem Res* **26**, 979-991.

[36] Cunha RA, Correia-de-Sá P, Sebastião AM, Ribeiro JA (1996) Preferential activation of excitatory adenosine receptors at rat hippocampal and neuromuscular synapses by adenosine formed from released adenine nucleotides. *Br J Pharmacol* **119**, 253-260.

[37] Wieraszko A, Goldsmith G, Seyfried TN (1989) Stimulation-dependent release of adenosine triphosphate from hippocampal slices. *Brain Res* **485**, 244-250.

[38] Cunha RA, Vizi ES, Ribeiro JA, Sebastião AM (1996) Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. *J Neurochem* **67**, 2180-2187.

[39] Pankratov Y, Lalo U, Verkhratsky A, North RA (2007) Quantal release of ATP in mouse cortex. *J Gen Physiol* **129**, 257-65

[40] Rebola N, Canas PM, Oliveira CR, Cunha RA (2005) Different synaptic and subsynaptic localization of adenosine A$_{2A}$ receptors in the hippocampus and striatum of the rat. *Neuroscience* **132**, 893-903.

[41] Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul JY, Takano H, Moss SJ, McCarthy K, Haydon PG (2005) Astrocytic purinergic signaling coordinates synaptic networks. *Science* **310**, 113-116.

[42] Mitchell JB, Lupica CR, Dunwiddie TV (1993) Activity-dependent release of endogenous adenosine modulates synap-

tic responses in the rat hippocampus. *J Neurosci* **13**, 3439-3447.

[43] Dunwiddie TV (1980) Endogenously released adenosine regulates excitability in the *in vitro* hippocampus. *Epilepsia* **21**, 541-548.

[44] Sebastião AM, Stone TW, Ribeiro JA (1990) The inhibitory adenosine receptor at the neuromuscular junction and hippocampus of the rat: antagonism by 1,3,8-substituted xanthines. *Br J Pharmacol* **101**, 453-459.

[45] Dixon AK, Widdowson L, Richardson PJ (1997) Desensitisation of the adenosine A$_1$ receptor by the A$_{2A}$ receptor in the rat striatum. *J Neurochem* **69**, 315-321.

[46] Lopes LV, Cunha RA, Ribeiro JA (1999) Crosstalk between A$_1$ and A$_{2A}$ adenosine receptors in the hippocampus and cortex of young adult and old rats. *J Neurophysiol* **82**, 3196-3203.

[47] Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. *Nature* **266**, 737-739.

[48] Serrano A, Haddjeri N, Lacaille JC, Robitaille R (2006) GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J Neurosci* **26**, 5370-5382.

[49] Manzoni OJ, Manabe T, Nicoll RA (1994) Release of adenosine by activation of NMDA receptors in the hippocampus. *Science* **265**, 2098-101

[50] Greene RW, Haas HL, Hermann A (1985) Effects of caffeine on hippocampal pyramidal cells *in vitro*. *Br J Pharmacol* **85**, 163-169.

[51] Cunha RA (2008) Cafeína, receptores de adenosina, memoria y enfermedad de Alzheimer. *Med Clin* **131**, 790-795.

[52] Takahashi RN, Pamplona FA, Prediger RD (2008) Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. *Front Biosci* **13**, 2614-2632.

[53] Ritchie K, Carrière I, de Mendonça A, Portet F, Dartigues JF, Rouaud O, Barberger-Gateau P, Ancelin ML (2007) The neuroprotective effects of caffeine: a prospective population study (the Three City Study). *Neurology* **69**, 536-545.

[54] Laitala VS, Kaprio J, Koskenvuo M, Räihä I, Rinne JO, Silventoinen K (2009) Coffee drinking in middle age is not associated with cognitive performance in old age. *Am J Clin Nutr* **90**, 640-646.

[55] Maia L, de Mendonça A (2002) Does caffeine intake protect from Alzheimer's disease? *Eur J Neurol* **9**, 377-382.

[56] Eskelinen MH, Ngandu T, Tuomilehto J, Soininen H, Kivipelto M (2009) Midlife coffee and tea drinking and the risk of late-life dementia: a population-based CAIDE study. *J Alzheimers Dis* **16**, 85-91.

[57] Lynch MA (2004) Long-term potentiation and memory. *Physiol Rev* **84**, 87-136.

[58] Govindarajan A, Kelleher RJ, Tonegawa S (2006) A clustered plasticity model of long-term memory engrams. *Nat Rev Neurosci* **7**, 575-583.

[59] Kemp A, Manahan-Vaughan D (2007) Hippocampal long-term depression: master or minion in declarative memory processes. *Trends Neurosci* **30**, 111-118.

[60] Remondes M, Schuman EM (2004) Role for a cortical input to hippocampal area CA1 in the consolidation of long-term memory. *Nature* **431**, 699-703.

[61] Izumi Y, Zorumski CF (2008) Direct cortical inputs erase long-term potentiation at Schaffer collateral synapses. *J Neurosci* **28**, 9557-9563.

[62] Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG (1998) Enhanced long-term potenti-

ation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433-439.

[63] Jolas T, Zhang XS, Zhang Q, Wong G, DelVecchio R, Gold L, Priestley T (2002) Long-term potentiation is increased in the CA1 area of the hippocampus of APP(swe/ind) CRND8 mice. *Neurobiol Dis* **11**, 394-409.

[64] Vaillend C, Billard JM, Laroche S (2004) Impaired long-term spatial and recognition memory and enhanced CA1 hippocampal LTP in the dystrophin-deficient Dmd(mdx) mouse. *Neurobiol Dis* **17**, 10-20.

[65] Sahún I, Delgado-García JM, Amador-Arjona A, Giralt A, Alberch J, Dierssen M, Gruart A (2007) Dissociation between CA3-CA1 synaptic plasticity and associative learning in TgN-TRK3 transgenic mice. *J Neurosci* **27**, 2253-2260.

[66] Ehninger D, Han S, Shilyansky C, Zhou Y, Li W, Kwiatkowski DJ, Ramesh V, Silva AJ (2008) Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. *Nat Med* **14**, 843-848.

[67] Muzzio IA, Levita L, Kulkarni J, Monaco J, Kentros C, Stead M, Abbott LF, Kandel ER (2009) Attention enhances the retrieval and stability of visuospatial and olfactory representations in the dorsal hippocampus. *PLoS Biol* 7, e1000140.

[68] Jensen O, Kaiser J, Lachaux JP (2007) Human gamma-frequency oscillations associated with attention and memory. *Trends Neurosci* **30**, 317-324.

[69] Hameleers PA, Van Boxtel MP, Hogervorst E, Riedel WJ, Houx PJ, Buntinx F, Jolles J (2000) Habitual caffeine consumption and its relation to memory, attention, planning capacity and psychomotor performance across multiple age groups. *Hum Psychopharmacol* **15**, 573-581.

[70] Hewlett P, Smith A (2007) Effects of repeated doses of caffeine on performance and alertness: new data and secondary analyses. *Hum Psychopharmacol* **22**, 339-350.

[71] Correia-de-Sá P, Timóteo MA, Ribeiro JA (1996) Presynaptic A_1 inhibitory/A_{2A} facilitatory adenosine receptor activation balance depends on motor nerve stimulation paradigm at the rat hemidiaphragm. *J Neurophysiol* **76**, 3910-3919.

[72] Ascherio A, Zhang SM, Hernán MA, Kawachi I, Colditz GA, Speizer FE, Willett WC (2001) Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Ann Neurol* **50**, 56-63.

[73] Ascherio A, Weisskopf MG, O'Reilly EJ, McCullough ML, Calle EE, Rodriguez C, Thun MJ (2004) Coffee consumption, gender, and Parkinson's disease mortality in the cancer prevention study II cohort: the modifying effects of estrogen. *Am J Epidemiol* **160**, 977-984.

[74] Xu K, Xu Y, Brown-Jermyn D, Chen JF, Ascherio A, Dluzen DE, Schwarzschild MA (2006) Estrogen prevents neuroprotection by caffeine in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neurosci* **26**, 535-441.

[75] Cunha RA, Almeida T, Ribeiro JA (2001) Parallel modification of adenosine extracellular metabolism and modulatory action in the hippocampus of aged rats. *J Neurochem* **76**, 372-382.