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Conditioning training and retrieval increase phospholipase A_2 activity in the cerebral cortex of rats

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Abstract In rats, phospholipase A_2 (PLA_2) activity was found to be increased in the hippocampus immediately after training and retrieval of a contextual fear conditioning paradigm (step-down inhibitory avoidance [IA] task). In the present study we investigated whether PLA_2 is also activated in the cerebral cortex of rats in association with contextual fear learning and retrieval. We observed that IA training induces a rapid (immediately after training) and long-lasting (3 h after training) activation of PLA_2 in both frontal and parietal cortices. However, immediately after retrieval (measured 24 h after training), PLA_2 activity was increased just in the parietal cortex. These findings suggest that PLA_2 activity is differentially required in the frontal and parietal cortices for the mechanisms of contextual learning and retrieval. Because reduced brain PLA_2 activity has been reported in Alzheimer disease, our results suggest that stimulation of PLA_2 activity may offer new treatment strategies for this disease.

Keywords Phospholipase A_2 · Rat · Frontal cortex · Parietal cortex · Learning · Retrieval

Introduction

Phospholipase A_2 (PLA_2) is a family of hydrolytic enzymes that catalyze the cleavage of fatty acids from the *sn*-2 position of membrane glycerophospholipids to generate lysophospholipids and free fatty acids (Dennis 1994, 1997). PLA_2-catalyzed hydrolysis of membrane phosphatidylcholine forms lysophosphatidylcholine and free arachidonic acid (AA), which are important mediators in signal transduction (Farooqui et al. 1997). The PLA_2 family is classified into three main groups: secretory (extracellular) Ca^{2+}-dependent PLA_2 ($sPLA_2$), cytosolic Ca^{2+}-dependent PLA_2 ($cPLA_2$), and intracellular Ca^{2+}-independent PLA_2 ($iPLA_2$) (Dennis 1994). The mRNA and/or activity of the three groups have been detected both in human (Chen et al. 1994; Larsson Forsell et al. 1999; Pickard et al. 1999; Gelb et al. 2000; Mancuso et al. 2000; Suzuki et al. 2000) and rat brains (Owada et al. 1994; Molloy et al. 1998; Kishimoto et al. 1999).

Previous studies from our laboratory showed reduced PLA_2 activity in *postmortem* parietal and frontal cortices of Alzheimer disease (AD) patients (Gattaz et al. 1995, 1996). These findings were supported by Ross et al. (1998), who reported reduced $cPLA_2$ and $iPLA_2$ activities in *postmortem* parietal and temporal cortices of AD patients, as well as decreased $cPLA_2$ activity in the hippocampus. Moreover, decreased $iPLA_2$ activity was found in *postmortem* prefrontal cortex of frontal-variant AD patients (Talbot et al. 2000).

Several studies in laboratory animals have shown that PLA_2 blockade impairs learning and memory, simulating deficits that are found since the earliest phases of AD and represent the most predominant cognitive changes in this disease. For instance, intracerebral infusion of non-selective PLA_2 inhibitors in chicks impaired learning of a passive avoidance task (Holscher and Rose 1994). Additionally, intraperitoneal injection of a non-selective PLA_2 inhibitor in rats impaired spatial learning tested in the Morris water maze (Holscher et al. 1995). Furthermore,

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intracerebroventricular infusion in mice of a non-selective PLA_2 inhibitor or a dual $cPLA_2$ and $iPLA_2$ inhibitor impaired memory formation of a step-through inhibitory avoidance task (in which a context, tone, and foot shock are presented together in an associative fashion) (Sato et al. 2007), and a selective $iPLA_2$ inhibitor impaired spatial learning tested in the Y-maze (Fujita et al. 2000). Recent studies from our group showed that infusion of dual $cPLA_2$ and $iPLA_2$ inhibitors or a selective $iPLA_2$ inhibitor into rat hippocampal CA1 field impaired acquisition of short- and long-term memory (Schaeffer and Gattaz 2005), and retrieval of long-term memory (Schaeffer and Gattaz 2007) of a contextual fear task (step-down inhibitory avoidance [IA], in which fear conditioning is induced by a single exposure to a context followed by an electric foot shock).

Memory training has been clinically performed and reported to be effective in improving memory function in elderly subjects with mild cognitive impairment (Rapp et al. 2002; Belleville et al. 2006; Wenisch et al. 2007) and early-stage AD (Clare et al. 2002; Abrisqueta-Gomez et al. 2004; Avila et al. 2004). Animal research has elucidated some possible brain biochemical mechanisms related to experience-dependent stimulation, and PLA_2 activation seems to be highly implicated here. For example, passive avoidance training was followed by enhanced concentration of AA (Clements and Rose 1996) and prostaglandins (cyclooxygenase products of AA metabolism) (Holscher 1995) in chick brains. Recent studies from our group showed that training of rats in the IA task increased the activity of endogenous PLA_2 in the hippocampal CA1 field (Schaeffer and Gattaz 2005). Additionally, our studies showed that re-exposure of rats to context after training (contextual memory retrieval) also stimulated PLA_2 activity in the CA1 field (Schaeffer and Gattaz 2007). In the present study we extended our previous findings in the hippocampus, by investigating the effects of contextual learning and retrieval on PLA_2 activity in the cerebral cortex of rats.

Materials and methods

One hundred and six male Wistar rats of 270–330 g (Central Animal Laboratory House, Federal University of São Paulo, Brazil) were used in the present study. All the procedures described were approved by the institutional animal ethics committee.

Inhibitory avoidance task

Step-down IA task was carried out as previously described (Vianna et al. 2000). The animals were placed on an 8.0 cm wide, 5.0 cm high platform at the left of a 50 cm wide, 25 cm deep, 25 cm high IA box (Albarsch, Brazil), whose floor was an electrified grid made of a series of parallel 1.0 mm caliber stainless steel bars spaced 1.0 cm apart. In training sessions, immediately after stepping down from the platform, placing the four paws on the grid, the rats received a 0.4 mA, 4.0 s scrambled foot shock. Latencies to step down were measured. Rats were tested for retrieval 24 h after training. In test sessions, the rats were allowed to stay on the platform up to a ceiling of 180 s, and no foot shock was given. Latencies to step down were measured. Test session step-down latency was taken as a measure of retention.

Three different experiments were carried out.

1. In the first experiment, 31 rats were divided into (a) trained animals: trained in the IA as described above and killed by decapitation immediately after training session; (b) naïve controls: killed by decapitation immediately after withdrawal from their home cages; and (c) shocked controls: placed directly over the electrified grid, given the foot shock, and immediately killed by decapitation. All trained animals stepped down the platform in the training session, showing a mean step-down latency of 9 ± 6 s.
2. In the second experiment, 38 rats were divided into (a) trained animals: trained in the IA and killed by decapitation 3 h after training session; (b) naïve controls: killed by decapitation immediately after withdrawal from their home cages; and (c) shocked controls: placed directly over the electrified grid, given the foot shock, and killed by decapitation 3 h later. All trained animals stepped down the platform in the training session, showing a mean step-down latency of 6 ± 4 s.
3. In the third experiment, 37 rats were divided into (a) trained/tested animals: trained in the IA, tested for retrieval 24 h later, and killed by decapitation immediately after retrieval test session; (b) naïve controls: killed by decapitation immediately after withdrawal from their home cages; and (c) trained controls: trained in the IA and killed by decapitation 24 h later. All trained animals stepped down the platform in the training session. Animals in the trained group showed a mean step-down latency of 8 ± 5 s, and animals in the trained/tested group showed a mean step-down latency of 7 ± 5 s. Animals in the trained/tested group were also tested for retrieval 24 h after training, showing a mean step-down latency of 112 ± 56 s.

Determination of PLA_2 activity

For PLA_2 activity determination, the rat brains were rapidly withdrawn and the frontal association cortex and

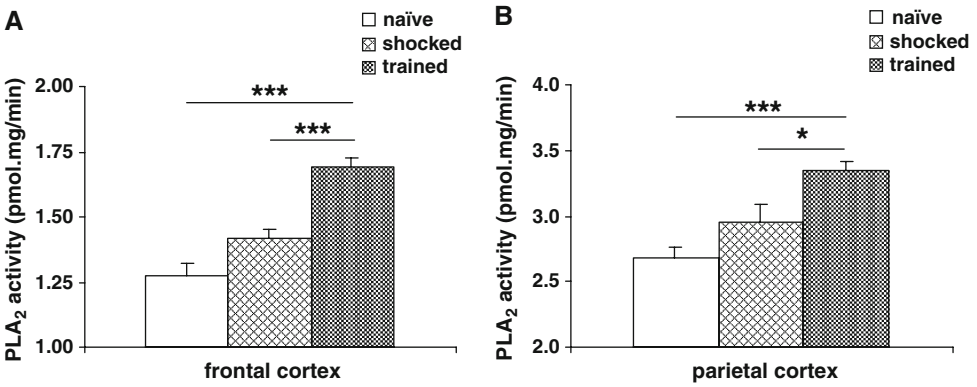

Fig. 1 PLA_2 activity measured immediately after training. PLA_2 activity (pmol mg protein min^{-1}) is given as mean (±SEM). **a** In the frontal cortex, PLA_2 activity was increased in trained animals ($n = 9$) by 32% as compared to naïve controls ($n = 8$), and by 20% as compared to shocked controls ($n = 10$). **b** In the parietal cortex, PLA_2 activity was increased in trained animals ($n = 10$) by 25% as compared to naïve controls ($n = 11$), and by 13% as compared to shocked controls ($n = 9$). Shocked and naïve controls had similar values of PLA_2 activity in both studies. $^*P < 0.05$, $^{***}P < 0.001$. P values were calculated using Bonferroni's test after ANOVA: Frontal cortex: $F_{(2,24)} = 26.57$, $P < 0.001$; Parietal cortex: $F_{(2,27)} = 14.03$, $P < 0.001$

parietal association cortex were bilaterally dissected according to visual anatomical landmarks and the Atlas of Paxinos and Watson (1998), and immediately stored at −70°C until use. The brain tissue was homogenized in 20 volume of 5 mM Tris–HCl buffer (pH 7.4, 4°C) and stored at −70°C. Prior to PLA_2 assay, total protein levels were determined for each aliquot by the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA, USA) modified from the Lowry assay (Lowry et al. 1951). PLA_2 activity was determined by a radioenzymatic assay, as previously described (Schaeffer and Gattaz 2005). Briefly, as enzyme substrate we used L-α-1-palmitoyl-2-arachidonyl-phosphatidylcholine labelled with [1-^{14}C] in the arachidonyl tail at the *sn*-2 position (arachidonyl-1-^{14}C-PC) (PerkinElmer, Boston, MA, USA). We used optimal assay conditions for measuring $cPLA_2$ plus $iPLA_2$ activity in rat brain homogenates, as previously determined by our group. Hence, the assay samples (500 µl) contained 50 mM Tris–HCl (pH 8.5), 1 µM $CaCl_2$, 300 µg of protein from homogenates, and 0.06 µCi arachidonyl-1-^{14}C-PC. After an incubation time of 30 min at 37°C, the radioactivity of the liberated [1-^{14}C]arachidonic acid was measured in a liquid scintillation counter (Tri-Carb 2100TR; Packard, Meriden, CT, USA) and used for calculating the PLA_2 activity, which is expressed in pmol mg protein min^{-1}. All determinations of PLA_2 activity were performed in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the values among groups in each time interval. Post hoc test consisted of the Bonferroni's multiple comparison test. Pearson correlation coefficient was calculated to determine the degree of association between PLA_2 activity and scores on the memory retrieval test of individual animals within the trained/tested group in the third experiment. Two-tailed probabilities < 0.05 were considered significant.

Results

PLA_2 activity measured immediately after training

Frontal association cortex PLA_2 activity was significantly increased in trained animals ($n = 9$) by 32% as compared to naïve controls ($n = 8$), and by 20% as compared to shocked controls ($n = 10$; $P < 0.001$). Shocked controls had similar values of PLA_2 activity as naïve controls ($P > 0.05$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,24)} = 26.57$, $P < 0.001$ (Fig. 1a).

Parietal association cortex PLA_2 activity was significantly increased in trained animals ($n = 10$) by 25% as compared to naïve controls ($n = 11$; $P < 0.001$), and by 13% as compared to shocked controls ($n = 9$; $P < 0.05$). Shocked controls had similar values of PLA_2 activity as naïve controls ($P > 0.05$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,27)} = 14.03$, $P < 0.001$ (Fig. 1b).

PLA_2 activity measured 3 h after training

Frontal association cortex PLA_2 activity was significantly increased in trained animals ($n = 11$) by 18% as compared to naïve controls ($n = 10$; $P < 0.01$), and by 13% as compared to shocked controls ($n = 11$; $P < 0.05$).

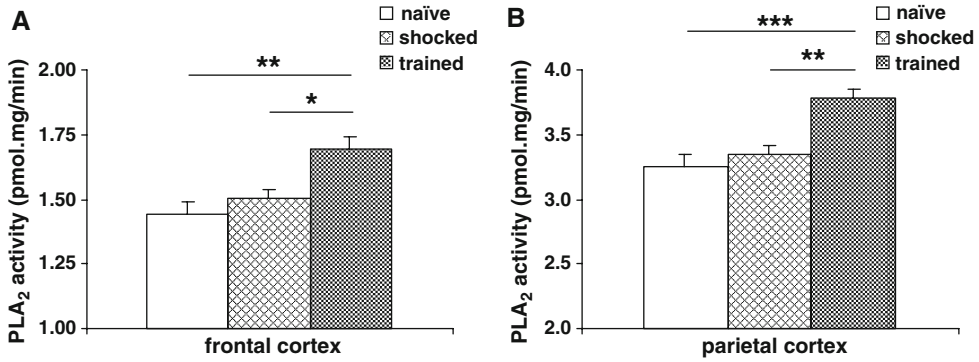

Fig. 2 PLA_2 activity measured 3 h after training. PLA_2 activity (pmol mg protein min^{-1}) is given as mean (±SEM). **a** In the frontal cortex, PLA_2 activity was increased in trained animals ($n = 11$) by 18% as compared to naïve controls ($n = 10$), and by 13% as compared to shocked controls ($n = 11$). **b** In the parietal cortex, PLA_2 activity was increased in trained animals ($n = 13$) by 16% as compared to naïve controls ($n = 13$), and by 13% as compared to shocked controls ($n = 12$). Shocked and naïve controls had similar values of PLA_2 activity in both studies. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. P values were calculated using Bonferroni's test after ANOVA: Frontal cortex: $F_{(2,29)} = 7.87$, $P < 0.01$; Parietal cortex: $F_{(2,35)} = 12.82$, $P < 0.001$

Shocked controls had similar values of PLA_2 activity as naïve controls ($P > 0.5$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,29)} = 7.87$, $P < 0.01$ (Fig. 2a).

Parietal association cortex PLA_2 activity was significantly increased in trained animals ($n = 13$) by 16% as compared to naïve controls ($n = 13$; $P < 0.001$), and by 13% as compared to shocked controls ($n = 12$; $P < 0.01$). Shocked controls had similar values of PLA_2 activity as naïve controls ($P > 0.5$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,35)} = 12.82$, $P < 0.001$ (Fig. 2b).

PLA_2 activity measured immediately after retrieval

Frontal association cortex Trained/tested animals ($n = 12$) had similar values of PLA_2 activity as naïve ($n = 11$; $P > 0.05$) and trained controls ($n = 14$; $P > 0.05$), and trained controls had similar values of PLA_2 activity as naïve controls ($P > 0.5$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,34)} = 3.29$, $P = 0.05$ (Fig. 3a).

Parietal association cortex PLA_2 activity was significantly increased in trained/tested animals ($n = 12$) by 27% as compared to naïve controls ($n = 11$; $P = 0.01$), and by 18% as compared to trained controls ($n = 14$; $P < 0.05$). Trained controls had similar values of PLA_2 activity as naïve controls ($P > 0.5$). P values were calculated using Bonferroni's test after ANOVA, $F_{(2,34)} = 6.24$, $P < 0.01$ (Fig. 3b).

Animals in the trained/tested group showed a mean step-down latency of 7 ± 5 s in the training session. These animals were also tested for retrieval 24 h after training,

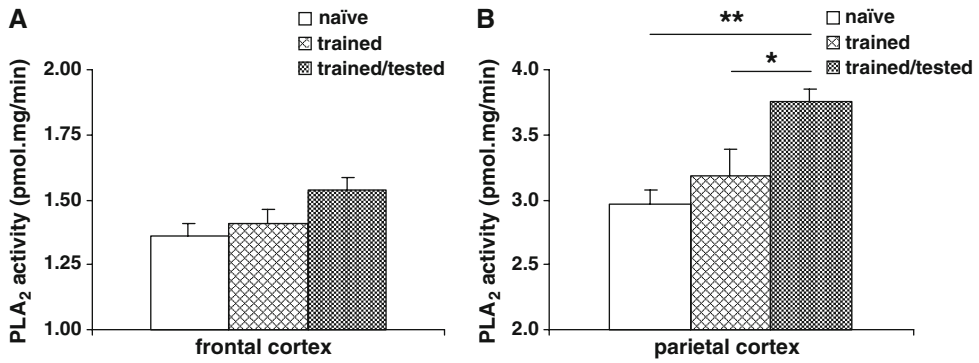

Fig. 3 PLA_2 activity measured immediately after retrieval. PLA_2 activity (pmol mg protein min^{-1}) is given as mean (±SEM). **a** In the frontal cortex, trained/tested animals ($n = 12$) had similar values of PLA_2 activity as naïve ($n = 11$) and trained controls ($n = 14$). **b** In the parietal cortex, PLA_2 activity was increased in trained/tested animals ($n = 12$) by 27% as compared to naïve controls ($n = 11$), and by 18% as compared to trained controls ($n = 14$). Trained and naïve controls had similar values of PLA_2 activity in both studies. $^*P < 0.05$, $^{**}P < 0.01$. P values were calculated using Bonferroni's test after ANOVA: Frontal cortex: $F_{(2,34)} = 3.29$, $P = 0.05$; Parietal cortex: $F_{(2,34)} = 6.24$, $P < 0.01$

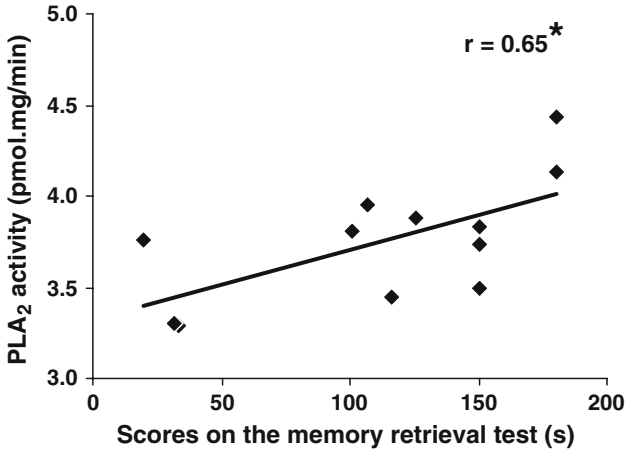

Fig. 4 Correlation between PLA_2 activity and scores on the memory retrieval test. PLA_2 activity (pmol mg protein min^{-1}) and scores on the memory retrieval test (i.e., test session step-down latencies, in seconds) are given as mean. Pearson correlation test showed a positive correlation between PLA_2 activity in the parietal cortex and scores on the memory retrieval test of rats within the trained/tested group ($n = 12$; $r = 0.65$, $^*P < 0.05$)

showing a mean step-down latency of 112 ± 56 s, that is 16-fold higher than the mean training session step-down latency. Pearson correlation test showed a positive correlation between PLA_2 activity in the parietal cortex and scores on the memory retrieval test (i.e., test session step-down latencies) of rats within the trained/tested group ($r = 0.65$, $P < 0.05$) (Fig. 4).

Discussion

In our previous studies (Schaeffer and Gattaz 2005, 2007) we found that PLA_2 activity was increased in the CA1 field of rat hippocampus immediately after training and retrieval of the step-down IA task. In the present study we extended our investigation to the cerebral cortex of rats, and found three major results. PLA_2 activity was increased in both frontal and parietal cortices of rats around the time of training and 3 h after training in the IA. However, PLA_2 activity was increased just in the parietal cortex of rats immediately after retrieval of the IA (Table 1). It should be noticed that, in both time intervals after training, PLA_2 activity was significantly increased in animals trained in the IA when compared to control animals that only received the electric foot shock (shocked controls) associated with the learning paradigm, indicating that increments in PLA_2 were specifically caused by the IA training. In the retrieval studies, we observed that the training effect on PLA_2 (in trained controls) disappeared after 24 h. However, the retrieval of the trained behavior in the IA task (in trained/tested animals) increased again the enzyme activity, indicating that increments in PLA_2 were specifically caused by the IA testing. Moreover, behavioral analysis revealed that animals in the trained/tested group showed a test session step-down latency in the IA 16-fold higher than the training session step-down latency, thus indicating good retention levels and that learning has occurred in this group. Most important, we found that increments in PLA_2 activity in the parietal cortex immediately after retrieval were highly correlated with scores on the memory retrieval test (i.e., test session step-down latencies) of rats within the trained/tested group. These findings support the suggestion that increments in PLA_2 activity immediately and 3 h after training were caused by learning. Altogether, the findings suggest that PLA_2 activity is differentially required in the frontal and parietal cortices of rats for the mechanisms of learning and retrieval of new contextual experience.

Experience-dependent changes have been extensively studied in rodent hippocampus and cerebral cortex in connection with contextual fear memory, and several biochemical mechanisms which are closely connected to

Table 1 Biochemical mechanisms involved in memory formation and retrieval of step-down inhibitory avoidance task in rats

	Memory formation							Memory retrieval
	Around training	30 min post-training	1 h post-training	1.5 h post-training	2 h post-training	3 h post-training	6 h post-training	
Frontal cortex	NMDA					NMDA		
	AMPA			AMPA		AMPA		
	PKC	PKC			PKC			
	PLA_2					PLA_2		
Parietal cortex			NMDA	NMDA		NMDA		NMDA
	AMPA							AMPA
								mGluR
	PKC	PKC			PKC	PKC	PKC	
	MAPK							MAPK
	PLA_2					PLA_2		PLA_2

PLA_2 have been implicated here. In the rat hippocampus, learning of the step-down IA was associated with elevations in the expression of NMDA NR1 subunit (Cammarota et al. 2000), increased activation of protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II), p38, p42 and p44 mitogen-activated protein kinase (MAPK), and increased [^{3}H]AMPA binding to the AMPA glutamate receptor (Cammarota et al. 1995, 1996, 1997, 1998; Bernabeu et al. 1995, 1997; Alonso et al. 2002, 2003). Learning of the step-through IA was also associated with increased activation of hippocampal PKC in rats (Young et al. 2002). Moreover, re-exposure of mice to context after training (contextual memory retrieval) stimulated the activity of hippocampal p42 and p44MAPK (Chen et al. 2005). Regarding the cerebral cortex, exposure of rats to the step-down IA resulted in increased activation of PKC in the frontal and parietal cortices at varying times after training (immediately, 30 min, and 2 h) (Bernabeu et al. 1995; Cammarota et al. 1997). Several pharmacological studies in rats have been conducted using the step-down IA, adding to the findings above. In the parietal cortex, blockade of NMDA receptors at varying times after training in the IA (1, 1.5 and 3 h) impaired memory consolidation (Zanatta et al. 1996; Izquierdo et al. 1997). In addition, blockade of AMPA receptors before or immediately after training in the IA disrupted memory acquisition and consolidation (Izquierdo et al. 1998). Furthermore, inhibition of MAPK activity immediately after training (Walz et al. 2000), and of PKC activity between 3 and 6 h after training in the IA impaired memory consolidation (Bonini et al. 2005). Finally, blockade of NMDA, AMPA and metabotropic glutamate receptors (mGluR), and inhibiton of MAPK impaired memory retrieval of the IA (Quillfeldt et al. 1996; Izquierdo et al. 1997; Barros et al. 2000). In the prefrontal cortex, blockade of NMDA receptors at different times after training in the IA (immediately and 3 h) impaired memory consolidation (Mello et al. 2000). Additionally, blockade of AMPA receptors before training or at varying times after training in the IA (immediately, 1.5 and 3 h) disrupted memory acquisition and consolidation, respectively (Izquierdo et al. 1998, 2007). Blockade of AMPA receptors before or immediately after training also disrupted memory of the step-through IA (Liang et al. 1996). Data on the cerebral cortex described in this paragraph are summarized in Table 1.

As already mentioned, PLA_2 activity is closely connected to all biochemical mechanisms described above. PLA_2-dependent release of AA is a receptor-mediated process. In this way, activation of postsynaptic NMDA receptors raises postsynaptic $[Ca^{2+}]i$ and stimulates $cPLA_2$, which generates AA, as found in mouse cortical neurons and rat hippocampal neurons and slices (Sanfeliu et al. 1990; Pellerin and Wolfe 1991; Lazarewicz et al. 1992; Stella et al. 1995). Many studies have demonstrated that PLA_2 can be regulated by a variety of protein kinases. For example, activation of $cPLA_2$ is regulated by PKC (Wijkander and Sundler 1991; Nemenoff et al. 1993), p38MAPK (Zhou et al. 2003), p42MAPK (Lin et al. 1993; Nemenoff et al. 1993; Gordon et al. 1996) and CaMKII phosphorylation (Muthalif et al. 2001), and $iPLA_2$ activation is regulated by PKC phosphorylation (Underwood et al. 1998; Akiba et al. 1999). In turn, stimulation of PLA_2 activity in the presence of Ca^{2+} in rodent cortical and hippocampal slices as well as membrane preparations increased [^{3}H]AMPA binding to the AMPA receptor and [^{3}H]glutamate binding to AMPA and mGluR (Massicotte and Baudry 1990; Baudry et al. 1991; Massicotte et al. 1991; Tocco et al. 1992; Catania et al. 1993; Bernard et al. 1995; Chabot et al. 1998; Gaudreault et al. 2004), whereas PLA_2 inhibition and the Ca^{2+} chelator EGTA reduced agonist binding to AMPA and mGluR (Bernard et al. 1993, 1995; Catania et al. 1993). Additionally, PLA_2 inhibition in rat hippocampal slices prevented Ca^{2+}-dependent formation of long-term potentiation (LTP; a synaptic model of learning and memory) in the CA1 field, as well as the increase of [^{3}H]AMPA binding to the AMPA receptor that characterizes LTP (Bernard et al. 1994). These findings support the involvement of $cPLA_2$-mediated AA release in learning and memory. AA has been suggested to be also released by activation of mGluRs. Selective blockade of the mGluR5 subunit inhibited LTP in the CA1 field of rat hippocampal slices, and AA administration restored LTP, suggesting that during LTP group I mGluRs cause AA release that may be mediated by stimulation of $iPLA_2$ (Izumi et al. 2000). Further studies support a role for $iPLA_2$ in LTP. Selective inhibition of the $iPLA_2$-VIB isoenzyme prevented LTP induction in the CA1 field of rat hippocampal slices, as well as the associated increase of [^{3}H]AMPA binding to the AMPA receptor and the up-regulation of AMPA GluR1 subunit levels in crude synaptic fractions (Martel et al. 2006). These findings support the involvement of $iPLA_2$-mediated AA release in learning and memory. Finally, AA potentiated the current through NMDA receptor channels in cerebellar granule cells, thus amplifying increases in $[Ca^{2+}]i$ caused by glutamate (Miller et al. 1992), and induced a long-lasting potentiation of AMPA receptor currents by increasing Ca^{2+} influx in *Xenopus* oocytes expressing AMPA receptors containing GluR1,3 subunits (Nishizaki et al. 1999).

It is noteworthy that $sPLA_2$ enzymes ($\sim$13–18 kDa) require millimolar $[Ca^{2+}]$ for catalytic activity (Farooqui et al. 1999), the activation of 85 kDa $cPLA_2$ is regulated by nanomolar or micromolar $[Ca^{2+}]i$ (Yoshihara and Watanabe 1990; Underwood et al. 1998), and $iPLA_2$ enzymes ($\sim$88 kDa) do not require Ca^{2+} for catalytic

activity (Larsson et al. 1998; Mancuso et al. 2000; Tanaka et al. 2000). We have previously optimized conditions for measuring $cPLA_2$ plus $iPLA_2$ activity or just $iPLA_2$ activity in rat brain homogenates (Schaeffer and Gattaz 2005). It was not possible to measure the activity of $cPLA_2$ alone, because $iPLA_2$ enzymes, which do not require Ca^{2+} for catalytic activity, can also respond to the optimal conditions for $cPLA_2$, i.e., nanomolar or micromolar $[Ca^{2+}]i$ (Larsson et al. 1998; Mancuso et al. 2000; Tanaka et al. 2000). In fact, in our previous study, using optimized conditions for $cPLA_2$ (micromolar $[Ca^{2+}]$ and pH 8.5), we found a dominant activity of $iPLA_2$, while $cPLA_2$ activity was about 11-fold lower than the $iPLA_2$ activity. Accordingly, Yang et al. (1999) reported a dominant $iPLA_2$ activity over $cPLA_2$ activity in the rat hippocampus as well as whole brain. However, despite the low activity of $cPLA_2$ in the rat brain, there is evidence for the involvement of $cPLA_2$ in the formation of LTP (Bernard et al. 1994; Weichel et al. 1999). Therefore, because both $cPLA_2$ and $iPLA_2$ have been implicated in mechanisms of synaptic plasticity and/or learning and memory, we applied in the present study the conditions for measuring $cPLA_2$ plus $iPLA_2$ activity previously determined (Schaeffer and Gattaz 2005). Considering the methodological limitations, the findings of the present study, taken together with previous studies described above, allow four major conclusions. In the parietal cortex, (1) activation of $cPLA_2$ and/or $iPLA_2$ around the time of training might modulate memory formation through up-regulation of AMPA receptors via a PKC (in the case of $cPLA_2$ and $iPLA_2$) and a MAPK-dependent pathway (in the case of $cPLA_2$); (2) activation of $cPLA_2$ and/or $iPLA_2$ 3 h after training might modulate memory formation through up-regulation of NMDA receptors via a PKC-dependent pathway; and (3) activation of PLA_2 (likely $cPLA_2$) around the time of testing might modulate memory retrieval through up-regulation of NMDA, AMPA, and mGluRs via a MAPK-dependent pathway. In the frontal cortex, (4) activation of $cPLA_2$ and/or $iPLA_2$ around the time of training and 3 h after training might modulate memory formation through up-regulation of NMDA and AMPA receptors via a PKC-dependent pathway. We are not aware of any study till date, showing an involvement of $sPLA_2$ in learning and/or memory. Thus, we did not look at $sPLA_2$ in the present study.

In the context of AD, where reduced PLA_2 activity has been reported in the frontal and parietal cortices (Gattaz et al. 1995, 1996; Ross et al. 1998; Talbot et al. 2000), the present findings could suggest that reduced PLA_2 activity in the parietal cortex of AD patients might contribute to impairment of context learning and memory retrieval. Regarding the reduced PLA_2 activity in the frontal cortex of AD patients, it might have a role in the impairment of context learning but not memory retrieval. Interestingly, a very recent study conducted by our group showed that cognitive training, consisting of a four-session memory training intervention for 1 month, increased PLA_2 activity in platelets of healthy elderly individuals, suggesting that memory training may have a modulating effect in PLA_2-mediated biological systems associated with cognitive functions (Talib et al. 2008). Because reduced PLA_2 activity has been reported in the frontal and parietal cortices of AD patients, and lower platelet PLA_2 activity was correlated with the severity of cognitive decline in samples of individuals with AD and mild cognitive impairment (Gattaz et al. 2004), the findings of the present study together with those of Talib et al. (2008) permit to speculate that stimulation of PLA_2 activity might offer new treatment strategies for the memory impairment seen in AD. Collectively, the data support the use of cognitive training as a promising non-pharmacological approach to stimulate PLA_2 at least in healthy elderly subjects for the prevention of cognitive deficits.

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