Training in the Morris Water Maze Occludes the Synergism Between ACPD and Arachidonic Acid on Glutamate Release in Synaptosomes Prepared from Rat Hippocampus

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Abstract

We report here that release of glutamate, inositol phospholipid metabolism, and protein kinase C (PKC) activity are increased in synaptosomes prepared from hippocampi of rats that had been trained in a spatial learning task. In hippocampi obtained from animals that were untrained, activation of the metabotropic glutamate receptor by the specific agonist *trans*-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) increased release of glutamate but only in the presence of a low concentration of arachidonic acid. A similar interaction between arachidonic acid and ACPD was observed on inositol phospholipid turnover and on PKC activity. However, the synergistic effect of arachidonic acid and ACPD on glutamate release was occluded in hippocampal synaptosomes prepared from trained rats. Occlusion of the effect on inositol phospholipid turnover and PKC activation was also observed. These data suggest that the molecular changes that underlie spatial learning may include activation of metabotropic glutamate receptors in the presence of arachidonic acid and that the interaction between arachidonic acid and ACPD triggers the presynaptic changes that accompany learning.

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Introduction

It is a widely held view that memory formation is dependent on changes in synaptic efficacy that allows strengthening (or weakening) of associations between neurons. The hippocampus has been identified as a brain area that plays a significant role in memory processing (Squire 1992), particularly processing of spatial memory (e.g., McNaughton et al. 1986; O'Keefe 1991), and though it has been extensively studied, the mechanisms that underlie acquisition and recall are not known. Certain cellular changes have been associated with learning; many of these have been identified as a result of comparison between young animals, which perform well in learning tasks, and aged animals, which often exhibit learning impairments (Barnes 1979), and in studies designed to investigate similarities and/or differences between learning and long-term potentiation (LTP). The hypothesis that LTP in the hippocampus is a biological correlate of learning and/or memory is actively challenged, but there is a good deal of indirect evidence lending support to this idea. Thus, it has been reported that AP5 blocks induction of LTP (see Bliss and Collingridge 1993) and also the ability of rats to perform in a spatial learning task (Morris et al. 1986), that both LTP and learning are associated with increased release of glutamate in dentate gyrus (Richter-Levin et al. 1994), and that protein kinase C (PKC) activity is increased following induction of LTP (Akers et al. 1986; Angenstein et al. 1994) and in a learning paradigm (Fordyce et al. 1994).

It has been reported recently that when acti-

vation of metabotropic glutamate receptors is blocked, learning in a spatial task is impaired (Richter-Levin et al. 1994), and this observation, as well as the finding that learning was accompanied by increased glutamate release in dentate gyrus, brings into the context of learning and recall the reports that *trans*-1-amino-cyclopentyl-1, 3-dicarboxylate (ACPD) and arachidonic acid (AA) act synergistically to increase release of glutamate in cortex (Herrero et al. 1992) and hippocampus (McGahon and Lynch 1994). AA has not yet been shown to play a role in spatial learning, but it has been implicated in learning in the chick (Hölscher and Rose 1994), and rat (Hölscher et al. 1995) and in maintenance of LTP in the dentate gyrus of the rat (Williams et al. 1989; Clements et al. 1991; Lynch and Voss 1994).

In designing these experiments, we considered that changes at the level of the synapse, resulting in “strengthening” of these synapses, probably accompany spatial learning and that presynaptic modifications are likely to contribute to these changes. In this study we have examined the effect of spatial learning on three parameters in hippocampal synaptosomes: glutamate release, inositol phospholipid metabolism, and PKC activation. In addition we have compared the effect of AA and ACPD, alone and in combination, on these three parameters in synaptosomes prepared from hippocampus obtained from untrained rats and hippocampus obtained from rats that had been trained to locate a hidden platform in the Morris water maze. The data presented indicate that spatial learning increases glutamate release, inositol phospholipid metabolism, and PKC activation. Moreover, although AA and ACPD interact to further increase all three measures in synaptosomes prepared from untrained tissue, this effect is occluded in hippocampus obtained from trained rats.

Materials and Methods

ANIMALS

Male Wistar rats (250–300 grams) were used in these experiments. Animals were housed in groups of four to six under a 12-hr light schedule. The temperature was controlled between 22°C and 23°C.

TRAINING SCHEDULE

A white fiberglass circular pool (diam., 120 cm; height, 48 cm; depth of water, 34 cm) was

used in these experiments. The platform (diam., 10 cm) was 4 cm below the water surface during training. The water was kept at $23 \pm 2^\circ\text{C}$ and made opaque with titanium dioxide. The pool was situated in a room with visual cues (e.g., door, window, curtain, etc.). All animals were marked with a black water-resistant marker to allow monitoring. The animals' movements were recorded with a video camera attached to the ceiling, and data was analyzed using a tracking program written by James Mahon (Trinity College, Dublin). During task acquisition, the program measured latency of animals to reach the platform and the distance covered. The pool was divided into four arbitrary quadrants for transfer test analysis. The quadrant in which the platform had been located (SE) was designated the target quadrant. During the transfer test, the percentage of the distance covered was assessed for each of the four quadrants.

Animals were placed in the water at one of four starting positions that alternated in a clockwise manner. Six trials were performed per day, and the intertrial interval was ~ 5 min. The cut-off time for a trial, if the animals failed to locate the platform, was 120 sec; in this case the animals were manually placed on the platform for 10 sec. On day 4, the platform was removed and the animals were given a 60-sec duration transfer test in the pool. The group that did not learn the task were given six swimming sessions in the pool each day without a platform. The duration of the session was the same as that of the group of animals that learned the task.

Animals were sacrificed by cervical dislocation 10–15 min after completion of the behavioral test. The hippocampus was dissected free; the time taken for this dissection was ~ 1 min. Slices (350 μm) were prepared using a McIlwain tissue chopper, suspended in Krebs solution, thoroughly mixed, and divided into three aliquots for later assessment of glutamate release, inositol phospholipid metabolism, and PKC activity. Slices were frozen in Krebs solution containing 10% DMSO (Haan and Bowen 1981) and stored in three separate aliquots in liquid N_2 until required for analysis.

TISSUE PREPARATION

Slices were thawed rapidly (1.5–2 min) by agitation at 37°C and rinsed four times in excess fresh oxygenated Krebs solution. Tissue was homogenized in 0.32 M ice-cold sucrose and centri-

fuged at 5000 rpm for 5 min. The crude synaptosomal pellet, P₂, was prepared by centrifuging the resulting supernatant at 15,000 rpm for 15 min. P₂ was used for analysis of glutamate release, inositol phospholipid metabolism, and PKC activity. All biochemical analysis was completed on all samples that were tested in the behavioral paradigm.

ASSESSMENT OF GLUTAMATE RELEASE

Synaptosomes were resuspended in ice-cold Krebs solution (composition in mM: NaCl, 136; KCl, 2.54; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.18; NaHCO₃, 16; glucose, 10) containing 2 mM CaCl₂ and incubated for 15 min at 37°C in the presence of [³H]glutamate (Amersham, UK; specific activity, 20–40 Ci/mmol; final concentration, 5×10^{-7} mM). Tissue was aliquoted onto Millipore filters (0.45 µm) and rinsed under vacuum at least 20 times by addition of 250 µl of ice-cold, oxygenated, fresh Krebs solution to the filter papers. Tissue was then incubated for 5 min at 37°C in 250 µl oxygenated Krebs solution with no added calcium or with Krebs solution containing 2 mM CaCl₂. The filtrate was discarded. This step was repeated, but incubation continued for 10 sec. [In earlier experiments, we investigated release at different incubation times (10, 20, and 60 sec and 5 min). Small time-related differences in the proportions of calcium-independent and calcium-dependent release were observed and because a greater proportion of calcium-dependent release was observed in the shorter time period, the 10-sec incubation period was chosen. This finding is consistent with the literature, which indicates that calcium-dependent release is initially very rapid, whereas calcium-independent release increases linearly with time (e.g., Nicholls 1989).] Filtrate was collected for scintillation counting. The incubation step was repeated, but in this case 40 mM KCl was added to depolarize the synaptosomes. In some cases AA (final concentration, 1 µM), ACPD (50 µM), or both were added during the incubation period to examine the effects of these agents on unstimulated and KCl-stimulated release. Results from previous experiments indicated that higher concentrations of AA increase release of glutamate in the absence as well as in the presence of calcium and also interfere with glutamate uptake (Lynch and Voss 1990); a final concentration of 1 µM AA was chosen in these experiments to avoid these con-

founding effects. In preliminary experiments, release of [³H]glutamate was compared in synaptosomes prepared in sucrose alone and in sucrose to which bovine serum albumin (BSA) was added to remove polyunsaturated fatty acid that may have been released during preparation. No difference between the two groups was observed, and, so, the experiments described here were performed on slices that were prepared in the absence of BSA.

ANALYSIS OF INOSITOL PHOSPHOLIPID TURNOVER

To examine [³H]inositol labeling of phosphoinositides and inositol phosphates, synaptosomes were resuspended in oxygenated ice-cold Krebs buffer (composition in mM: NaCl, 136; KCl, 2.54; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.18; NaHCO₃, 16; CaCl₂, 1.3; glucose, 10; cytidine, 1; LiCl, 5) and aliquots (50 µl) were incubated at 37°C for 35 min in a shaking water bath in 120 µl Krebs buffer containing [³H]myo-inositol (final concentration, 0.3 µM; specific activity, 110 mCi/mg; Amersham, UK). AA (10 µl; final concentration, 1 µM) or ACPD (10 µl; final concentration, 50 µM) or an equivalent volume of control vehicle was added to the synaptosomal suspension at that point, and incubation continued for a further 35 min. In some experiments, both ACPD (final concentration, 50 µM) and AA (final concentration, 1 µM) were present in the incubation medium. The final assay volume was 250 µl in all experiments. The reaction was terminated by addition of ice-cold trichloroacetic acid (18 µl; final concentration, 5%), 940 µl of chloroform/methanol 2:3 (vol/vol), 310 µl of chloroform, and 310 µl of water. Organic and aqueous layers were vortex mixed and separated by centrifugation. To assess [³H]inositol labeling of inositol phosphates, aliquots (400 µl) of the aqueous phase were added to 1 ml of Dowex slurry (formate form, 50% in water; Dowex-1, 8% cross-linked, 200 mesh, Sigma) and washed four times with myo-inositol (5 mM). Inositol phosphates were eluted with 500 µl of 0.1 M NH₄OH in 0.1 M formic acid. Aliquots (400 µl) of the eluate were added to scintillation fluid for scintillation counting and calculation of total labeling of inositol phosphates by [³H]inositol. To assess labeling of phosphoinositides by [³H]inositol, aliquots (320 µl) of the lower organic phase were evaporated overnight, scintillant was added, and samples counted for radioactivity. The protein

concentration was estimated by the method of Bradford (1976), and all values were expressed as cpm/ μ g protein.

ANALYSIS OF PKC

PKC activity was examined using an enzyme assay system (Amersham, UK) that assessed transfer of the radiolabeled phosphate group from adenosine-5-triphosphate ($[^{32}\text{P}]\text{ATP}$) to histone, a peptide specific for PKC. P_2 was prepared and lysed by incubating at 4°C for 10 min in lysis buffer [composition: 5 mM Tris-HCl at pH 7.5, containing 5 mM EDTA, 10 mM EGTA, 0.3% (wt/vol) β -mercaptoethanol, 10 mM benzamidine, and 50 $\mu\text{l}/\text{ml}$ of phenylmethylsulphonyl fluoride]. The membrane fraction was pelleted and resuspended in assay buffer [composition: 50 mM Tris-HCl at pH 7.5, containing 5 mM EDTA, 10 mM EGTA, 0.3% (wt/vol) β -mercaptoethanol, 10 mM benzamidine and 50 $\mu\text{l}/\text{ml}$ of phenylmethylsulphonyl fluoride]. The reaction was started by addition of $[^{32}\text{P}]\text{ATP}$ to the synaptosomal suspension, which was then incubated at 25°C for 15 min. In some experiments, the effect of AA (final concentration, 1 μM), ACPD (50 μM), or both was assessed; in this case the compounds were added prior to the 15-min incubation period. The final assay volume was 75 μl . Phosphorylated peptide was separated by applying the reaction mixture to binding paper and washing with 5% (vol/vol) acetic acid. Binding papers were added to scintillant and ^{32}P counted. Results were expressed as picomoles phosphate transferred per minute.

STATISTICAL ANALYSIS

A one-way analysis of variance was performed to determine whether there were significant differences between conditions. When this analysis indicated significance (at the 0.05 level), post hoc Student's Newmann Keuls test analysis was used to determine which conditions were significantly different from each other. In some cases the Student's *t*-test for independent means was used to establish statistical significance.

MATERIALS

ACPD was obtained from Tocris (UK); AA and other standard chemicals were obtained from

Sigma (UK). All radiochemicals and the PKC assay kit were obtained from Amersham (UK).

Results

TRAINING

The animals that were trained in the water maze task learned the task in the acquisition trials. The period of time required to locate the platform was reduced from 102 sec in trial 1 to 8 sec in trial 18 (Fig. 1). In the transfer task, untrained control animals, which swam in the pool during the acquisition phase without a platform, did not show a preference for any quadrant (ANOVA over all quadrants, $F(3,20) = 2.4$, $P > 0.05$; $n = 6$). In contrast, animals that were trained, spent a greater proportion of time in the SE quadrant (the target quadrant) than in any of the other quadrants (ANOVA over all quadrants, $F(3,20) = 21$, $P < 0.001$; $n = 6$). A two-tailed unpaired *t*-test showed a significant difference in the distance swum in the SE quadrant by the trained group compared with the untrained control group ($t = 3.09$ with 10 degrees of freedom; $P < 0.02$). Within the trained group, there was a significant difference in the distance swum in the target quadrant (SE) compared with the distance swum in the opposite quadrant (NW quadrant; $t = 7.1$ with 10 degrees of freedom; $P < 0.001$; $n = 6$).

Figure 1: (A) Time required to locate a hidden platform in a water maze. The period of time required to locate the platform was reduced from 102 sec in the first trial to 8 sec in the eighteenth trial. (B) Percentage of path swum by rats in a transfer task without a platform. Trained rats swam a greater distance in the target (SE) quadrant compared with the opposite (NW) quadrant ($P < 0.001$). Trained rats also swam a significantly greater distance in the target quadrant than untrained rats ($P < 0.02$).

EFFECTS OF AA AND ACPD ON GLUTAMATE RELEASE

Mean accumulation of [^3H]glutamate was similar in tissue prepared from rats that were untrained and those that were trained in the Morris water maze ($12,285 \pm 983$ vs. $12,854 \pm 1794$, respectively). Mean unstimulated release was also similar in synaptosomes prepared from trained and untrained rats ($0.096\% \pm 0.008$ and $0.11\% \pm 0.007$, respectively). Addition of 40 mM KCl to the incubating medium significantly increased glutamate release in the absence of calcium, but a further significant increase was observed in the presence of calcium (data not shown). Figure 2A shows that KCl-stimulated glutamate release (i.e., release in the presence of 40 mM KCl minus unstimulated release) in the presence of calcium was significantly greater in hippocampal synaptosomes prepared from trained rats compared with untrained rats ($P < 0.05$; Student's *t*-test for independent means). Neither AA ($1 \mu\text{M}$) nor ACPD ($50 \mu\text{M}$) alone had any significant effect on release in hippocampal synaptosomes prepared from untrained rats ("control synaptosomes"), but AA and ACPD together induced a significant increase in release ($P < 0.001$; ANOVA). As in the case of control synaptosomes, neither AA nor ACPD alone had any significant effect on release of glutamate in synaptosomes prepared from tissue obtained from trained rats. However, in contrast to the effect in control synaptosomes, there was no evidence of the synergism between AA and ACPD on release in synaptosomes prepared from hippocampus obtained from trained rats (Fig. 2A).

EFFECT OF AA AND ACPD ON INOSITOL PHOSPHOLIPID METABOLISM

Labeling of total inositol phospholipids with [^3H]inositol was used as an index of inositol phospholipid turnover. [^3H]inositol labeling of phosphoinositides was similar in synaptosomes prepared from trained and untrained rats, and no significant effect of AA, ACPD, or both together was observed (data not shown). Figure 2B shows that inositol phospholipid turnover was significantly increased in synaptosomes prepared from hippocampus of trained rats compared with untrained rats ($P < 0.05$; Student's *t*-test for independent means). In control synaptosomes, both AA ($1 \mu\text{M}$) and ACPD ($50 \mu\text{M}$) significantly increased inositol

phospholipid turnover ($P < 0.05$ in each case; ANOVA); the increase was significantly greater in the presence of both AA and ACPD ($P < 0.01$ compared with control; $P < 0.05$ compared with AA alone or ACPD alone; ANOVA). In synaptosomes prepared from tissue obtained from trained rats, neither AA nor ACPD alone nor both agents together had any significant effect on inositol phospholipid metabolism (Fig. 2B).

EFFECT OF AA AND ACPD ON PKC ACTIVITY

Figure 2C demonstrates that activity of PKC was significantly enhanced in synaptosomes prepared from tissue obtained from trained rats compared with control ($P < 0.001$; Student's *t*-test for independent means). AA ($1 \mu\text{M}$) and ACPD ($50 \mu\text{M}$) alone significantly enhanced PKC activity in "control" tissue ($P < 0.05$ in each case) but when both AA and ACPD were included in the incubation medium, a further increase was observed ($P < 0.001$ compared with control; $P < 0.05$ compared with AA alone or ACPD alone; ANOVA). In contrast to the observations in control synaptosomes, neither AA nor ACPD had any significant effect on PKC activity in synaptosomes prepared from hippocampus of trained rats. Moreover, the interaction between AA and ACPD on PKC activity, which was clearly evident in control tissue, was absent in these synaptosomes (Fig. 2C).

Discussion

The hypothesis that memory formation requires modification at the level of the synapse is central to the growing number of investigations designed to identify the mechanisms underlying learning and/or memory. In the present experiments we examined one specific form of learning, that is, training in the Morris water maze, and investigated the possibility that this leads to changes in the presynaptic terminal in hippocampus thereby contributing to an alteration in synaptic strength. The results show enhanced release of glutamate, inositol phospholipid metabolism, and PKC activity in synaptosomes prepared from animals that had undergone training in a spatial learning task. We also report that the synergism between AA and ACPD on these three measures, which we observe in hippocampi obtained from

pared from trained rats, compared with untrained rats (+ $P < 0.05$; Student's *t*-test for independent means); but no effect of AA, ACPD, or both agents together was observed in hippocampal synaptosomes prepared from trained rats. Values are means ($\pm$ S.E.M.) of six observations and are expressed as picomoles phosphate incorporated/min.

untrained rats, was occluded in hippocampi obtained from trained rats.

The present data indicate that a low concentration of AA did not affect glutamate release, supporting earlier reports (Herrero et al. 1992; McGahon and Lynch 1994) and contrasting with the inhibitory effect of higher concentrations (Lynch and Voss 1990). The finding that ACPD had no effect on release indicates that it is unlikely to activate a presynaptic glutamate receptor that functions as an autoreceptor, although such a receptor has been observed in neonates (Baskys 1992). Although no effect of either AA or ACPD alone was observed, these agents acted synergistically to increase glutamate release in synaptosomes prepared from hippocampi of untrained animals, supporting earlier observations in the cortex (Herrero et al. 1992), whole hippocampus

(McGahon and Lynch 1994), and dentate gyrus (McGahon and Lynch 1996). This effect was absent in hippocampal synaptosomes prepared from trained rats. This observation suggests that training occluded this effect, although it must be considered that other factors, for example, stress, may have contributed to the changes. Because ACPD is a nonspecific activator of mGluRs, it is not possible to speculate on the receptor subtype activated in these experiments. However, because previous evidence suggests that coupling to cAMP is unlikely (McGahon and Lynch 1994) and because PLC activation is stimulated by ACPD, it might be speculated that mGluR1 or mGluR5 may play a role (Pin and Duvoisin 1995).

The increase in release of glutamate described here supports the recent observation of Richter-Levin et al. (1995) who reported that training in a

spatial learning task was associated with increased glutamate release in dentate gyrus. In the present study, tissue was prepared 15 min after the transfer test, whereas in the study of Richter-Levin, tissue was prepared 4 hr after testing, and, therefore, it is possible that recall contributes to a greater extent to the changes described in the present study. It is of interest that classical conditioning has also been associated with increased glutamate release in dentate gyrus (Laroche et al. 1987). The fact that increased glutamate release is a feature of both learning and maintenance of LTP (Lynch et al. 1989; Canevari et al. 1994) supports the idea that LTP may be a biological substrate for learning and memory. Further support of this nature is presented here, because we have found that the synergism between AA and ACPD on glutamate release in dentate gyrus was occluded by prior induction of LTP in this area (McGahon and Lynch 1996).

In an effort to establish the mechanisms underlying the AA/ACPD-induced increase in glutamate release, we examined the effect of these agents on inositol phospholipid metabolism, an increase that leads to formation of inositol trisphosphate (IP_3) and diacylglycerol. Both second messengers have the ability to increase transmitter release, by increasing intracellular calcium concentration and PKC activity, respectively. The data presented indicate that training in the Morris water maze led to an increase in inositol phospholipid metabolism in whole hippocampus. Changes in inositol phospholipid metabolism in behavioral paradigms have been reported previously; associative learning is accompanied by an increase in basal inositol phospholipid metabolism in the three subfields of the hippocampus (Laroche et al. 1990), while training in an eight-arm radial maze is accompanied by an increase in ibotenate-induced, but not basal, inositol phospholipid metabolism (Nicoletti et al. 1986). We report here that both AA and ACPD increased inositol phospholipid metabolism, as described previously in the case of AA (Lynch et al. 1988; Lynch and Voss 1990) and as described in other brain areas in the case of ACPD (Schoepp et al. 1990; Pin and Duvoisin 1995). In addition to these responses, we found that AA and ACPD interacted to further increase inositol phospholipid metabolism, confirming our previous observation (McGahon and Lynch 1994). In parallel with our release data, we found that the stimulatory effects of AA and ACPD, alone and in combination, were occluded in synaptosomes prepared

from trained animals. These parallel effects suggest that the increase in glutamate release may derive from increased inositol phospholipid metabolism.

It is well established that IP_3 increases intracellular calcium concentration in several systems, including in synaptosomes prepared from dentate gyrus (Lynch and Voss 1991), and this may contribute to release; activation of PKC also stimulates transmitter release (Parfitt and Madison 1993; Terrian et al. 1993). One interpretation of these results is that the increase in glutamate release observed here is a consequence of increased inositol phospholipid metabolism. The occlusion of the effects of AA and ACPD on inositol phospholipid metabolism by training in the water maze is similar to the occlusion observed following induction of LTP (McGahon and Lynch 1996), lending indirect support to the idea that the mechanisms underlying spatial learning resemble those underlying maintenance of LTP.

Both AA and ACPD alone significantly enhanced PKC activity in synaptosomes prepared from hippocampus obtained from untrained rats, and the combination of the two agents induced a further increase in whole hippocampus as described previously (McGahon and Lynch 1994). In parallel with the occlusion of the AA/ACPD synergism on release and inositol phospholipid metabolism by training, the data indicate that the interaction between AA and ACPD on PKC is also occluded. Training also occluded the separate actions of AA and ACPD on PKC. The close coupling in responses of phospholipase C and PKC to AA and ACPD, both alone and in combination, suggests that PKC activation may be secondary to phospholipase C activation. AA has been shown to activate PKC (Murakami and Routtenberg 1985), but the present results suggest that this may be secondary to an effect on inositol phospholipid metabolism.

There is a good deal of evidence suggesting that activation of PKC plays a role in spatial learning. Thus, physical activity that increases performance in a spatial learning task also increases PKC activity (Fordyce et al. 1994). In addition, PKC activity in hippocampus of aged mice (C57) with learning impairments was found to be reduced compared with aged mice (F_1) in which no age-related learning impairment was observed (Fordyce and Wehner 1993). It has also been shown that performance in the Morris water maze was correlated positively with PKC substrate phosphorylation (Wong et al. 1989). It is of interest

that increased activation of PKC following induction of LTP has been reported (Akers et al. 1986; Angenstein et al. 1994; Pasinelli et al. 1995), as well as an increase in presynaptic PKC activity in area CA1 (Leahy et al. 1993). Although indirect, the parallel responses in PKC activity following a learning paradigm and induction of LTP, provide further support for the notion that LTP is a biological substrate for learning and/or memory.

One interpretation of the data presented here is that spatial learning induces changes in inositol phospholipid metabolism leading to PKC activation, and that these changes, in turn, lead to an increase in glutamate release. The inference is that this sequence of events will occur as a consequence of mGluR activation in the presence of AA. However, it is possible that training modifies release of other transmitters and that these may impact on glutamate release.

Our proposal is that learning induces changes presynaptically that lead to increased glutamate release and therefore a strengthening of certain synapses. Our data suggest that the increase in release may derive from increased inositol phospholipid metabolism and thence increased PKC activation. Because the effects of AA and ACPD, alone and together, are occluded in synaptosomes prepared from trained animals, it is tempting to conclude that an interaction between these compounds lies at the heart of the changes induced by training and therefore plays a pivotal role in the process of synaptic strengthening.

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