Correlations between hippocampal protein kinase C activity and learning abilities in a spatial reference memory task

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Partial learning of a reference memory task induces a decrease in hippocampal cytosolic protein kinase C (PKC) activity with no concomitant changes in the particulate fraction. The particulate PKC activity is correlated with performance (Noguès, Micheau, & Jaffard, 1994). The relationship between the level of training and PKC activity was investigated in the present study. Hippocampal PKC activity was measured after mice were trained for 2, 5, or 12 sessions in a spatial discrimination task in an 8-arm radial maze. No increase in membrane-bound fraction or decrease in cytosolic PKC activity was observed at any stage of learning, although performance was correlated with PKC activity in each subcellular fraction. We sought correlations between performance and the magnitude of the decrease in cytosolic PKC activity, with reference to previously obtained data. The extent of the fall in cytosolic PKC was found to be related to learning abilities rather than to the maximal performance level. Causal explanatory models are proposed to explain these somewhat contradictory data.

The biophysical substrate of memory in the brain is thought to reside in specific distributions of modifiable synaptic weights that establish internal representations in neural networks (Lisman, 1989; McNaughton & Morris, 1987). Long-term changes in the efficacy of cell-to-cell communication are assumed to be a reflection of persistent biochemical and morphological changes that could constitute the physiological bases of the information-encoding process (Alkon & Naito, 1986; Dudai, 1989). In line with this idea, artificial intelligence studies have shown that complex cognitive abilities may emerge from an association of formal neurons exhibiting plasticity in organized networks. Such cognitive abilities include the recognition of patterns (Minsky & Papert, 1990; Rosenblatt, 1962) and the resolution of spatial problems (Hopfield, 1986).

Glimpses into the mechanisms underlying plasticity suggest that cognitive functions could perhaps be improved by pharmacological manipulations. Considerable effort has thus been expended in the search for biochemical processes that produce persistent activity-dependent alterations in synaptic connections.

Since the discovery of long-term potentiation (LTP) of synaptic transmission in the hippocampus by Bliss and Lomo (1973), much research has focused on the molecular mechanisms underlying this phenomenon. It is as-

sumed that similar kinds of mechanisms are involved in learning and memory. For example, the glutamatergic NMDA channel is now thought to play a key role in the induction, but not maintenance, of LTP (Collingridge & Bliss, 1987; Errington, Lynch, & Bliss, 1987). The influx of calcium during induction of LTP may trigger a cascade of biochemical events leading to a persistent increase in synaptic excitability. NMDA channels have also been shown to be involved in acquisition but not retention processes (Staubli, Thibault, DiLorenzo, & Lynch, 1989). Among the events involved in both LTP and memory, the activation of protein kinase C (PKC) may play a key role. Application of PKC inhibitors before LTP induction accelerates the decay of LTP (Klann, Chen, & Sweatt, 1991; Linden & Routtenberg, 1989; Lovinger, Wong, Murakami, & Routtenberg, 1987), whereas its activation induces a potentiation (Lovinger & Routtenberg, 1988). Since PKC is highly concentrated in the hippocampus (Ito et al., 1990; McGinty, Couce, Bohler, & Ways, 1991), it may well play an important part in the neurobiological processes underpinning memory.

It is of interest that alterations in the distribution of protein kinase C have been observed in the hippocampus following different types of learning (Bank, DeWeer, Kuzirian, Rasmussen, & Alkon, 1988; Noguès et al., 1994; Olds, Anderson, McPhie, Staten, & Alkon, 1989; Olds et al., 1990; Paylor, Morrison, Rudy, Waltrip, & Wehner, 1992). An increase in membrane-bound PKC activity along with a decrease in soluble PKC activity has been observed after Pavlovian conditioning (Bank et al., 1988). Olds et al. (1990) found a decrease in (³H)phorbol-dibutyrate binding following both spatial and cued discrimination learning in the Morris water maze, although there was an increase in this binding following classical

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conditioning (Olds et al., 1989). Paylor et al. (1992) evidenced an increase in the cytosolic fraction of this enzymatic activity following 12 days of exposure to an enriched environment.

More recently, we have shown that learning of a spatial reference memory task in a radial maze induces a decrease in hippocampal cytosolic PKC activity with no concomitant changes in the membrane-bound fraction (Noguès et al., 1994). Nevertheless, membrane-associated PKC activity was found to be correlated with performance.

There are some discrepancies between our findings and those of other researchers. For instance, unlike Bank et al. (1988), we did not observe any increase in the membrane-bound fraction. The rabbits of Bank et al. had reached a high level of training when they were sacrificed, whereas our mice were sacrificed at the early stage of learning. On the other hand, we noted a positive correlation between the magnitude of the membrane-associated activity and the number of reference memory errors (Noguès et al., 1994). This correlation should be abolished if further training leads to an increase in membrane-bound PKC activity.

The first study presented in this paper was designed to test whether an increase in the level of training would lead to a cumulative increase in membrane-bound PKC activity across sessions and thus abolish the previously observed correlation. These results led us to examine in a second set of experiments (second study) the relationships between the amplitude of the decrease in cytosolic PKC activity and the ability to learn the task.

GENERAL METHOD

Animals and Maintenance

Male mice of the inbred strain BALB/c by Jico were used in the experiments. At 10 to 11 weeks of age they were housed individually with ad-lib access to food and water in a temperature-controlled room ($23 \pm 1^\circ\text{C}$) with a 12:12-h light:dark cycle (7 h on; 19 h off). The animals were 14 to 18 weeks old at the start of the experiments. Behavioral testing was performed in the morning.

Apparatus and Behavioral Testing

Behavioral testing was conducted in an automated elevated 8-arm radial maze described in detail elsewhere (Marighetto, Durkin, Toumane, Lebrun, & Jaffard, 1989). The experimental animals were trained in a reference memory task with a food reward. Two days before the first training session, the animals were progressively food deprived to maintain their body weight at 88% of their free-feeding weight throughout the experimental period.

The behavioral procedure has been described elsewhere (Noguès et al., 1994). Briefly, testing began with a habituation procedure during which the animals were allowed to explore freely the radial maze on 2 successive days. During this stage, all the doors of the maze were left open so that the mice could freely enter any arm and find a food pellet reward. Each daily session was terminated when all eight arms had been visited. Over the following days, the animals were trained in the spatial discrimination task. The test was arranged so that only one arm was baited, always the same for each mouse. Acquisition of discrimination consisted in learning the location of this arm. At the start of each trial, the eight doors were opened and the trial was terminated when the animal had found the food pellet. The doors of the other arms were closed and the last door was closed when the mouse had returned to the

central platform. The next trial began less than 30 sec later. Eight trials were performed daily, and this set of eight trials was called a "training session." The reference memory errors (first entries into a nonbaited arm) were recorded.

Biochemistry

Preparation of cytosolic and particulate fractions. We used the method described in Noguès et al. (1994) with slight modifications. Mice were rapidly sacrificed and hippocampi were dissected on ice within 5 min after decapitation. All subsequent procedures were carried out at 4°C . Both hippocampi from every mouse were homogenized in 500 μl of 30 mM Tris HCl buffer (pH 7.4) containing 2 mM EDTA and 0.32 M sucrose. The homogenate was centrifuged at $100,000 \times g$ for 30 min. The resulting supernatant (S2), termed the cytosolic fraction, was removed and stored. The pellet (P2) was resuspended in the same buffer but containing 0.05% (vol/vol) Triton X100. This particulate fraction was solubilized by sonication for 5 min and incubated for 30 min at 4°C prior to centrifugation at $100,000 \times g$ for 30 min. The new supernatant (S3) was termed the particulate fraction. Supernatants S2 and S3 were collected for determination of PKC activity.

Protein kinase C activity. PKC activity was determined by measuring the incorporation of ^{32}P from (γ - ^{32}P)ATP into histone-type IIIS in the presence of either EGTA (a calcium chelator) or calcium and PKC activators. For the first study, protein kinase C activity was measured with a slightly modified version of the method of Wehner, Sleight, and Upchurch (1990). The reaction mixture (100 μl) contained 20 mM Tris HCl pH 7.4, 25 mM magnesium acetate, 500 $\mu\text{g}/\text{ml}$ of histone-type IIIS, (γ - ^{32}P)ATP (0.1 μCi , New England Nuclear) and either 2.5 mM EGTA or 2.5 mM CaCl_2 , 4 $\mu\text{g}/100 \mu\text{l}$ of phosphatidyl serine and 0.4 $\mu\text{g}/100 \mu\text{l}$ of oleoyl 1-2 acetyl glycerol.

After initiation of the reaction by addition of 20 μl of membrane or soluble extract solution, the mixture was incubated for 5 min at 30°C . The reaction was stopped by applying 40- μl aliquots in duplicate on 3×3 cm squares of Whatman P81 paper. The squares were then immersed in 15 mM tetrasodium diphosphate and 10% trichloroacetic acid for 7 min. This immersion was repeated and the squares were then rinsed for 40 min under running tap water. The squares were air-dried and the associated radioactivity was determined in a liquid scintillation counter. Calcium-dependent protein kinase and PKC activity was taken as the difference between the activities in the presence and absence of Ca^{++} /phosphatidylserine. Protein content was determined according to the method of Bradford (1976). Protein kinase activity was expressed as nmoles of phosphorylated histones/min/mg of proteins.

For the second study in this paper, we employed the modified method of Wehner et al. (1990) described above and the modified method of Kitano et al. described in a previous study (Noguès et al., 1994).

FIRST STUDY

These experiments were designed to test whether an increase in the level of training led to an increase in membrane-bound PKC activity accompanying a decrease in cytosolic PKC activity, and whether this affected the correlation between membrane-bound PKC activity and performance.

Method

Experimental procedure. Animals were randomly divided into four groups: quiet control mice that were maintained in their home cages until sacrifice ($n = 12$), and three experimental groups that were trained daily. The first experimental group performed 2 training sessions ($n = 9$); the experimental procedure described in

Figure 1. Decrease in reference memory errors across sessions.

a previous study was followed (Noguès et al., 1994). One group performed 5 training sessions ($n = 9$) and the third performed 12 sessions ($n = 10$).

Animals were sacrificed 24–26 h following the last training session. The membrane-bound and cytosolic PKC activities of these three experimental groups were compared to those of the quiet control group. PKC activity was measured as described above (see General Method).

Statistics. Data are presented as the mean $\pm$ SEM. PKC activities for each group were compared by analysis of variance (Statview 512+).

Correlations between protein kinase activity and performance were established (Pearson correlation), followed by appropriate significance tests. For the correlations between membrane-bound PKC and performance, a one-tail correlation coefficient test was used. We attempted to verify the positive correlation between the number of reference memory errors and membrane-bound PKC activity. Since no hypotheses were made about the correlation between cytosolic PKC or calcium-independent protein kinase activity and performance, two-tail correlation coefficient tests were used. These simple correlation tests were complemented with partial correlation analyses when necessary.

Results

Behavioral results. Learning curves are presented in Figure 1. Taken together, the three experimental groups exhibited a significant reduction in the number of reference memory errors between the first and second sessions [$t(27) = 2.59$, $p = .008$, one-tail paired Student t test]. However, the level reached on the second training session was below that attained in our previous study

(19.8 ± 1.18 in Noguès et al., 1994; against 24.0 ± 1.36 in this experiment).

Concerning the 5-training-session group, the decrease in reference memory errors was continuous from the first to the last session [$F(4,32) = 17.5$, $p < .001$].

Finally, the 12-training-session group exhibited a significant increase in performance (i.e., a decrease in reference memory errors) from the first session to the seventh [$F(6,54) = 18.6$, $p < .001$]. Thereafter, there was no further progression in the six final sessions [$F(5,45) = 1.32$, $p = .27$].

Calcium-independent protein kinase activity. There was no significant difference in calcium-independent kinase activities in either cytosolic or particulate fractions between any of the four groups [respectively, $F(3,36) = 0.59$, $p = .62$; $F(3,36) = 0.036$, $p = .99$; see Table 1]. Moreover, no correlations were found between calcium-independent kinasic activity and performance ($-.22 < r < +.39$).

Calcium- and phospholipid-dependent protein kinase activity. As shown in Table 1, there were no significant effects of training on either cytosolic or membrane-bound PKC activity [respectively, $F(3,36) = 0.24$, $p = .87$, and $F(3,36) = 0.55$, $p = .65$], and no significant correlations between cytosolic or membrane-bound PKC activity and performance on the second training session (respectively, $r = .17$ and $r = .05$).

To simplify the results, the following learning stages have been grouped into two blocks, one composed of the mean of the number of reference memory errors from the third to the fifth session (3 to 5 block), and the other from the sixth to the last session (6 to 12 block).

Membrane-bound PKC activity was correlated with the mean number of reference memory errors in the 3 to 5 block ($r = .53$, $df = 17$, $p = .01$, one-tail test), but the correlation in the 6 to 12 block did not reach statistical significance ($r = .46$, $df = 8$, n.s., one-tail test; Figure 2).

Surprisingly, the results also showed a positive correlation between cytosolic PKC activity and the mean number of reference memory errors from the third to the fifth session ($r = .54$, $df = 17$, $p = .02$, two-tail test), but the correlation was not significant for the 6 to 12 block ($r = .59$, $df = 8$, n.s., two-tail test).

The lack of a significant correlation between the performance on the last block (Y) and both cytosolic (X) or membrane-bound (X') PKC activities may have been

Table 1
Mean and Standard Error of Kinasic Activity (nmol/min/mg Protein)

Group	Calcium-Independent Kinasic Activity				Calcium- and Phospholipid-Dependent Kinasic Activity			
	Membrane		Cytosol		Membrane		Cytosol	
	M	SE	M	SE	M	SE	M	SE
Controls	0.340	0.072	0.314	0.079	1.62	0.37	5.43	0.94
2 sessions	0.353	0.162	0.342	0.066	1.46	0.32	5.63	0.67
5 sessions	0.346	0.160	0.373	0.091	1.40	0.40	5.56	0.88
12 sessions	0.358	0.095	0.348	0.139	1.59	0.58	5.29	0.99

Figure 2. Correlation between membrane-bound or cytosolic protein kinase C (PKC) activity in the hippocampus, and the number of reference memory errors.

due to a floor effect or to the low sample size. However, since performance on the second (Z) and last blocks were strongly correlated, a rigorous analysis of these correlations must neutralize this correlation before the real correlation between PKC activity and performance on the 6 to 12 block is calculated. The results of the partial correlation analysis showed that $r_{XYZ} = .12$ for cytosolic, and $r_{XYZ} = -.09$ for membrane-bound PKC activity. On the other hand, the same analysis performed on the first sessions and on the 3 to 5 block did not alter (or slightly increased) the correlation between PKC activity and performance on the second block (respectively, $r = .61$ instead of $.59$, and $r = .53$ instead of $.48$). These partial correlation analyses indicate that both cytosolic and membrane-bound PKC activities were correlated with the number of errors recorded on a phase during which performance was increasing (i.e., to the rapidity of the acquisition of the task).

In summary, this experiment failed to replicate the fall in cytosolic PKC activity previously observed following the first two training sessions (Noguès et al., 1994), although it does confirm the correlation between membrane-bound PKC activity and the number of reference memory errors despite the lack of correlation of PKC activity with performance obtained in the second session. In fact, this lack of correlation is not altogether surprising, as performance was close to the chance baseline in the second training session. Thus, a correlation between enzymatic activity and the chance level of performance would have indicated that the former was correlated with a non-specific component of behavior such as stress, frustration,

or locomotor activity. Of more interest from the partial correlation analyses is the finding that the membrane-bound PKC activity was correlated with the ability to learn the task.

What is more troubling is that cytosolic PKC activity was also correlated with the rapidity of acquisition of the task, although we did not observe any significant decrease in cytosolic PKC activity. In a previous study, the same behavioral task led in some cases to a decrease in cytosolic PKC activity (Noguès et al., 1994), although the amplitude of the decrease induced by this task was rather variable (unpublished data). Despite the lack of statistically significant changes in PKC activity, it is tempting to suggest that a slight and statistically undetectable translocation occurred in the highest performers. The amplitude and the direction of the change may thus influence learning or covary with the ability to learn.

SECOND STUDY

The first set of experiments indicated that the decrease in cytosolic PKC is not strictly reproducible. Although the decrease in the number of reference memory errors is significant when the animals from this new experiment are included, performance in the second session was higher in the experiments reported in a previous publication. Thus, as suggested by the first set of experiments presented here, the decrease in cytosolic PKC activity may be related to the ability to learn the task. The second study was thus designed to test the hypothesis that the amplitude of the decrease in cytosolic PKC activity is positively correlated with performance in the early stage of learning.

We have now collected a considerable body of data on the changes in cytosolic PKC activity during this behavioral procedure. These data, along with those of the second set of experiments, were thus combined to test the correlation between these two parameters.

Method

Animals. From the whole set of data collected, only those derived from BALB/c mice ($n = 77$) were selected for this analysis (see General Method for animal care). The animals were sacrificed 5 min, 1 h, or 24 h following the last training session. The details about these mice are listed in Table 2. We have previously demonstrated that the interval between the last session and sacrifice does not have a significant effect on the decrease in cytosolic PKC activity over this time range (Noguès et al., 1994).

Training procedure. The behavioral testing procedure has been described above. However, as shown in Table 2, the animals were submitted to different numbers of training sessions.

Biochemistry. Two different methods were used to assay PKC activity. Wehner et al.'s (1990) modified method is described in the General Method section of this paper. The modified method of Kitano, Go, Kikkawa, and Nishizuka (1986) is described elsewhere (Noguès et al., 1994). An *in vitro* translocation of PKC induced by aniracetam was carried out to compare the results of the two methods. Both methods demonstrated a decrease in cytosolic PKC activity and an increase in membrane-bound PKC activity.

Data standardization. Cytosolic PKC activity was expressed as the percentage of the basal activity represented by the cytosolic

Table 2
Details of Animals Used in the Analysis

Experiment Identification	No. of Learning Sessions	Interval Between Last Session and Sacrifice	Biochemical Method	No. of Animals
1	2	5 min	Kitano et al.	5
1	2	1 h	Kitano et al.	6
1	2	24 h	Kitano et al.	6
2	2	5 min	Kitano et al.	3
2	2	1 h	Kitano et al.	2
2	2	24 h	Kitano et al.	3
3	4	1 h	Kitano et al.	5
4	2	24 h	Wehner et al.	9
4	5	24 h	Wehner et al.	9
4	12	24 h	Wehner et al.	10
5	2	24 h	Kitano et al.	4
6	2	5 min	Kitano et al.	3
6	2	1 h	Kitano et al.	2
6	2	24 h	Kitano et al.	1
7	2	5 min	Kitano et al.	3
7	2	1 h	Kitano et al.	3
7	2	24 h	Kitano et al.	3

PKC activity of the quiet controls kept in their home cages. The mean cytosolic PKC activity of the quiet controls was determined for each session of PKC quantification. The individual values for each trained animal were then divided by the basal level determined beforehand and expressed as percentages.

Results

A one-tail Student *t* test showed that the cytosolic PKC activity of the trained animals was significantly lower than the 100% basal level [$t(76) = 4.75, p < .001$].

There was a significant correlation between the number of reference memory errors recorded in the second training session and the percentage of cytosolic PKC activity ($r = .25, df = 75, p = .015$). In contrast, there was no correlation between the percentage of cytosolic PKC activity and performance in the session just preceding sacrifice ($r = -.14, df = 75, p = \text{n.s.}$). Cytosolic PKC activity thus appeared to be correlated with learning abilities but not with the degree of mastery of the task (Figure 3).

This pattern of correlations may indicate that the level of performance reached at the end of training does not determine the amplitude of the decrease in cytosolic PKC activity. Since PKC activity is correlated with an early step of training, irrespective of the number of sessions performed, this activity may be more related to the ability to learn the task.

GENERAL DISCUSSION

In the first set of experiments we investigated whether an increase in the level of training would lead to an increase in membrane-associated PKC activity and examined the effects of overtraining on the correlation found between membrane-bound PKC activity and performance (Noguès et al., 1994). In accordance with the latter data, this activity appears to be correlated with performance obtained at a stage of learning when the number of errors is declining rapidly. Cytosolic PKC activity also

appears to be correlated with performance at the same stage of learning. Both membrane-bound PKC activity and cytosolic PKC activity may thus be correlated with the ability to learn the task rather than with the degree of mastery of the task.

Our data did not replicate the decrease in cytosolic PKC at any stage of learning, even in the group of animals treated under the same conditions as those in the previous paper. We also failed to show any increase in the membrane-bound fraction. Since we did not observe any decrease in cytosolic PKC activity, we cannot conclude that there is no increase in membrane-bound PKC activity after a high level of training. Nevertheless, in view of the relative slowness of acquisition and the new-found correlation between performance and cytosolic PKC activity, we suggested that the amplitude of the decrease in cytosolic PKC activity was also linked to the ability to learn. The second set of experiments was designed to test this hypothesis on a much larger data set. This analysis revealed a small but significant correlation between the rapidity of acquisition and the amplitude of the decrease in cytosolic PKC activity, although no correlations were found between this enzymatic activity and the level of performance reached just before sacrifice.

Taken together with the data in the previous paper, the following comments can be made on these results. First, the decrease in cytosolic PKC activity may be an early phenomenon during learning, at least in our behavioral paradigm. Moreover, since it does not occur either in partially trained, well-trained, or overtrained animals, it might not be cumulative across sessions. In other words, the maximum decrease for each animal might be reached at an early stage of learning.

This new finding is in conflict with observations on the change in PKC conformation during the course of a behavioral task. Beldhuis, Everts, Van Der Zee, Luiten,

Figure 3. Correlation between the number of reference memory errors (second session) and the magnitude of the decrease in cytosolic protein kinase C (PKC) activity (percentages).

and Bohus (1992) demonstrated an increase in γ PKC immunostaining in the hippocampus across sessions of a spatial reference memory task in the hole-board maze. However, Van Der Zee, Compaan, De Boer, and Luiten (1992) showed that the application of phorbol ester on fixed tissues of naive control animals led to an increase in immunoreactivity resembling the increase observed in well-trained animals. According to the model of Huang and Huang (1993), five conformations representing five levels of PKC activity may be found *in vivo*. Sustained training may thus lead to changes in the conformation of PKC without inducing any alteration in overall activity. It would be of interest to find out whether there are changes in this activity after extraction of PKC in the absence of calcium chelators.

The results of Paylor et al. (1992) are more difficult to reconcile with our own results. They showed that brief exposure to an enriched environment led to an increase in the cytosolic fraction of the PKC activity after 12 but not 6 days of exposure to the environment. However, Olds et al. (1990) failed to show any changes in phorbol

ester binding following transient exposures to the Morris water maze, and Beldhuis et al. (1992) only showed slight modifications in immunostaining following repetitive exploration of the hole-board maze.

The origin and implications (in terms of causal relationships) of the correlation of performance with the fall in cytosolic PKC activity and level of membrane-bound PKC activity remain to be elucidated. As shown in Figure 4, this set of results presents a great analogy with the data obtained by Jeffery and Morris (1993).

We have previously shown that certain types of spatial learning (place learning tasks) sometimes induce a detectable decrease in cytosolic PKC activity, whereas a more general knowledge of the environment does not (Noguès et al., 1994). In the present study we found that the extent of the decrease in cytosolic PKC activity was variable.

With regard to the PKC activation process, a large fall in cytosolic PKC activity may be indicative of strong physiological activation of this enzyme. A translocation of this molecule from the cytosol to the membrane, fol-

Figure 4. Results obtained by Jeffery and Morris (1993) show that high-frequency stimulations (Event 1) of the perforant path lead to a long-term potentiation (LTP) (Event 2) of synapse efficiency. The authors induce LTP to an asymptotic level, whose amplitude exhibits individual differences (Event 3). This amplitude is correlated with subsequent performance (Event 4) in the Morris water maze. Moreover, induction of LTP does not modify (Event 5) performance. Thus, it is not LTP per se that covaries with learning, but rather another parameter such as prior individual synaptic excitability (Event 6).

In the case of protein kinase C (PKC) activity, we have shown that learning (Event 1') of a spatial reference memory task leads to a decrease in cytosolic PKC (Event 2') whose amplitude exhibits individual differences (Event 3'). This amplitude is correlated with performance (Event 4') in the radial maze.

Thereafter, the pattern of description is slightly different. Since an increase in level of training does not modify (Event 5') the amplitude of the decrease in PKC activity, this amplitude is not dependent on performance level. Rather, learning abilities may covary with prior PKC activation capability (Event 6').

In conclusion, these observations and relationships indicate that some preexisting properties of neural systems predetermine learning abilities. To explain the observed correlations, two causal models may be put forward. The linear one, whereby Event 6 or 6' may predict learning abilities (Event 4 or 4'), is most tempting. However, the intervention of common factors determining both of these events (6 or 6' and 4 or 4') cannot be ruled out.

lowed by its limited proteolysis by calpains, may lead to a soluble calcium-independent form (protein kinase M).

Studies using an interventionist approach have shown an impairment of learning after administration of PKC inhibitors (Ali, Bullock, & Rose, 1988; Burchuladze, Potter, & Rose, 1990; Mathis, Lehmann, & Ungerer, 1992; Takashima, Yokota, Maeda, & Shinji, 1991), whereas PKC activators have been shown to improve performance (Laborit & Zerbib, 1987; Paylor, Rudy, & Wehner, 1991). As suggested (Noguès et al., 1994), the involvement of hippocampal PKC may be task dependent. Pharmacological data suggest that the correlation between the fall in cytosolic PKC activity and learning abilities is of the causal type. For instance, nootropic drugs have been found to induce both in vitro and in vivo a translocation of PKC activity from the cytosol to the membranes (i.e., a decrease in the cytosolic fraction and an increase in the membrane-bound fraction), and then a decrease in the membrane-associated fraction (Lucchi, Pascale, Battaini, Govoni, & Trabucchi, 1993; Pascale et al., 1994). In other words, the property of the hippocampal system to trigger intense PKC activation may determine, at least in part, the ability to learn that kind of task. The results of Paylor et al. (1992), showing an increase in the cytosolic PKC activity, point to an adaptive mechanism dedicated to further specific training rather than a direct involvement in the acquisition of knowledge of the complex environment. Moreover, an adaptive mechanism is more likely to be required in a complex environment than in the home cage. In this viewpoint, these authors suggested that animals that have been exposed for 12 days to the enriched environment should have more PKC to translocate during the learning of subsequent tasks, such as those requiring spatial information.

The results also raise the problem of the functional role of the hippocampus in learning and memory. The difference in the influence of associative or nonassociative tasks on hippocampal PKC behavior suggests an involvement of this structure in associative processes. Moreover, data obtained in our laboratory have shown an impairment of learning by intrahippocampal inhibition of PKC and an improvement of long-term retention by its activation (Noguès, Micheau, & Jaffard, 1992, 1993), indicating that activation of hippocampal PKC plays a role in this kind of learning. In spite of the fact that these data argue in favor of a functional involvement of hippocampus in a reference memory task in the radial maze, they do not prove that this structure is absolutely necessary to learn this task. However, the deficit induced by hippocampal lesions in the spatial reference memory version of the Morris water maze task provides a strong argument for the requirement of this structure (Morris, 1988). Additional experiments are needed, however, to confirm this hypothesis in the particular task we have used.

From our previous results, we surmised that membrane-bound and cytosolic PKC belong to different pools. Because membrane-bound PKC activity did not change, whereas cytosolic PKC activity decreased, they appear to

behave independently. Although our data provide only indirect evidence for the existence of two different pools of PKC (i.e., particulate stock and soluble stock), more direct evidence has emerged from biochemical studies (Orr, Yavin, & Lester, 1992). Moreover, because membrane-bound PKC activity was not affected by training, we suggested that the level of this activity is set before the task. In the present study, we also observed a negative correlation between membrane-bound PKC and learning abilities. However, we assumed that membrane-bound PKC (especially the PKC remaining on the membranes during extraction with EGTA) is capable of phosphorylating its substrates (Bazzi & Nelsetuen, 1988; Huang & Huang, 1993). This pool may swamp the amount of cytosolic PKC translocated transiently during learning. This would lower the ratio between transiently membrane-associated activity (i.e., efficient activity) and permanent basal activity, thereby reducing the efficiency of the process. Another hypothesis is that the permanent membrane-bound PKC activity blocks some membrane-associated receptors for PKC. Mochly-Rosen and colleagues (Mochly-Rosen, Khaner, & Lopez, 1991; Mochly-Rosen, Khaner, Lopez, & Schmitt, 1991) have identified such receptors, termed RACKs (Receptors for Activated C Kinase), which are thought to be specific and saturable. Permanent membrane-bound PKC may thus limit the effects of newly translocated PKC. Nevertheless, firm evidence for accepting or rejecting these interpretations has yet to be obtained.

An interesting possibility is that cytosolic and membrane-bound PKC pools are constituted by different isozymes and that the different isozymes have different functions. If this is the case, slight modifications in a specific PKC activity during the task may be masked by the activity of the other isozymes. This could account for the paradox of a lack of statistically significant decrease in cytosolic PKC, but a correlation with performance.

As noted, there is a lack of consensus in the data on the involvement of PKC in learning and memory. This is not altogether unexpected in view of the complexity of the PKC activation processes in vivo (cf. Huang & Huang, 1993), the different methodologies employed to assay PKC, and the range of behavioral paradigms involving different cognitive processes used. However, by indicating an explanation for the lack of systematic reproducibility, the data presented in this paper highlight how such a paradox can contribute to the understanding of the complexity of the mechanisms involved.

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