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Changes in PKCγ Immunoreactivity in Mouse Hippocampus Induced by Spatial Discrimination Learning

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In the present study, we examined changes in immunoreactivity (ir) for the γ-isoform of protein kinase C (PKCγ) in mouse hippocampus in relation to spatial memory processes employing the monoclonal antibody 36G9 raised against purified PKCγ. Learning and memory were assessed by performance in a free-choice spatial pattern paradigm in a hole board in which the animals learned the pattern of 4 baited holes out of 16 holes. Adult male house mice were used, divided in four groups. Three control groups were formed: group N, naive (blank controls); group H, habituated (animals were for 5 consecutive days introduced to the hole board with all holes baited); and group PT, pseudotrained (animals were for 13 consecutive days introduced to the hole board with all holes baited). The T (trained) group was for 5 consecutive days introduced to the hole board with all holes baited (similar to the H and PT groups) followed by 8 successive days with only four holes baited in a fixed pattern. Behaviorally, following the first 5 d, the PT group crossed the hole board randomly, whereas the T group gradually learned to orientate in the hole board. The mice were killed 24 hr after the last performance. A shift in 36G9-ir appeared from the cell somata to the dendrites of hippocampal principal neurons when comparing the H and PT group, respectively. In contrast, the T group showed strong PKCγ-ir in both cell somata and dendrites, which clearly exceeded that of the H and PT mice. In this way, 36G9-ir reveals the physiologically activated neurons involved in hole board learning. The present results, showing changes in PKCγ-ir and redistribution of hippocampal PKCγ induced by hole board learning, are consistent with the observation that PKC is involved in spatial memory processes.

Protein kinase C (PKC) is a key enzyme for signal transduction and various neuronal plasticity mechanisms, and can be activated by receptor-stimulated turnover of phosphoinositides (Nishizuka, 1986, 1988; Strosberg, 1991). Rat brain PKC consists of seven distinct isoforms, each with a characteristic distribution within the CNS (K.-P. Huang et al., 1986; Kikkawa et al., 1987; F. L. Huang et al., 1988; Saito et al., 1988; Stichel and Singer, 1988; Hosoda et al., 1989; Kose et al., 1990; McGinty et al., 1991). PKC is active in the membrane-associated form and has been implicated in long-term cellular regulation including the formation and maintenance of different types of memory in the brain (Akers, 1986; Kennedy, 1988; Burgoyne, 1989; Messing et al., 1989; Olds et al., 1989). Activated PKC has been associated with long-term potentiation, a phenomenon that correlates with learning and memory (Hu et al., 1987; Anwyl, 1989; Linden and Routtenberg, 1989). The hippocampal formation is a critical neuronal substrate in learning and memory processes, notably in associative and spatial discrimination learning (Olton and Papas, 1979; McNaughton et al., 1986; Schmajuk, 1990; Nadel, 1991). Behaviorally induced changes in the distribution of PKC play an important role in associative memory storage within the hippocampus (Olton et al., 1991). Such changes in PKC distribution have been reported for the rabbit hippocampus after Pavlovian conditioning (Bank et al., 1988; Scharenberg et al., 1991). An increase in PKC content demonstrated by radioactive phorbol ester binding was primarily localized in the hippocampal pyramidal cell somata 24 hr after conditioning, but shifted to the basilar dendrites 48 hr later (Olds et al., 1990). Furthermore, mouse hippocampal PKC activity correlated positively with the ability to learn a spatial discrimination in a Morris water maze (Wehner et al., 1990a,b). Activation of PKC by intracerebroventricular injections of phorbol ester improved spatial learning in rats (Paylor et al., 1991). These results indicate that hippocampal PKC activity is an essential step for a successful performance in spatial memory tasks.

However, the above-mentioned studies did not discriminate between PKC isozymes. Furthermore, the limited anatomical resolution of the techniques used did not allow the study of the type of cells involved in the PKC redistribution. Since PKCγ appeared to be the most abundant isozyme in the hippocampus (F. L. Huang et al., 1988; Yoshida et al., 1988), we aimed to examine the contribution of PKCγ to hippocampal spatial memory processes. To answer this question, we employed the monoclonal antibody 36G9 raised against purified PKCγ (Cazaubon et al., 1989, 1990). 36G9 applied to hippocampal brain sections of mice trained in spatial navigation in a hole board enabled us to study the hippocampal cell types and cellular structures displaying changes in PKCγ distribution.

Part of this study has been reported in preliminary form elsewhere (Luiten et al., 1991).

Materials and Methods

In the present study, 26 adult male house mice (*Mus musculus domesticus*) of a custom breed were used. Mice of this strain were previously demonstrated to be good performers in a problem-solving maze (Benus

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et al., 1987). All 26 animals were individually housed in a sound- and light-attenuated experimental room on a 12 hr light/dark cycle. The lights were on between 24:30 and 12:30. The mice used for testing were food deprived until they reached 85–90% of their body weight under free feeding conditions. The animals were fed at approximately 17:30 hr with a quantity of food adjusted to maintain the food-deprived body weight until the end of the experiment. The test for spatial orientation was performed in the same room in the first 5 hr of the dark period (between 12:30 and 17:30). The animals were divided into four groups: group N, naive (n = 6); group H, habituated (n = 6); group PT, pseudotrained (n = 6); and group T, trained (n = 8). Group N was neither food deprived nor tested and served as blank controls. The animals of all other groups were food deprived throughout the experiment. During the first 3–4 d of habituation to the hole board, their body weight gradually reduced to 85–90% of their free feeding values. This body weight was maintained throughout the experiment.

The hole board is a test apparatus for spatial orientation (Oades and Isaacson, 1978; Oades, 1981). The hole board (70 × 70 × 45 cm) contained 16 equidistant holes (14 cm apart, 3.5 cm diameter, 3 cm depth) in the floor plate, as described by Oades (1981). All holes contained food pellets at the bottom on which a replaceable, perforated false bottom was placed. Thus, the mice were unable to discriminate between baited and nonbaited holes by orientating on olfactory cues in the hole board. Before testing, the animals were placed in a start box attached to one of the walls of the hole board. After 10 sec, the guillotine door between the start box and the arena of the hole board was lifted, allowing the mouse to enter the arena. A hole visit was scored when the nose of the mouse was placed in it. For habituation, the mice belonging to the H, PT, and T groups spent two trials of 3 min each on 5 consecutive days in the hole board with food in all holes in order to habituate and to get used to visiting holes to eat food. Between the trials, the floor of the start box and the hole board arena was cleaned with a wet and dry cloth.

Group H was killed after the habituation period. Groups PT and T were exposed to two trials per session on 8 successive days. For group PT, all holes were baited on the 8 consecutive days as a prolonged habituation. For group T, a fixed set of four holes arranged in a symmetrical pattern were supplied with an accessible food pellet (according to Oades, 1981). The mice of group T were removed from the hole board after either all four food holes were visited or a total testing time of 3 min per trial. As a consequence of learning, the average time spent in the hole board gradually decreased per trial from 180 sec at days 1–5 (the habituation period) to 171, 152, 147, 128, 118, 116, 111, and 112 sec at days 6, 7, 8, 9, 10, 11, 12, and 13, respectively. The total time the PT animals were allowed to spend in the hole board was adjusted to that of group T.

The reference memory ratio (RMR) was defined as (number of visits and revisits to the baited set of holes) ÷ [(4 − number of visits to baited holes) + (number of visits and revisits to nonbaited holes + number of revisits to baited holes)] of the first trial. The score of RMR ranges from 0 (no baited holes visited) to 1 (only the four baited holes visited). Student's t test (correlated samples) was used to analyze the data. The RMR scores on days 7–13 were compared with the RMR score on day 6.

Immunocytochemical procedure. Twenty-four hours after the last introduction (H group), last prolonged introduction (PT group), or last trial (T group), the animals were, similar to the animals of the N group, deeply anesthetized with 6% sodium pentobarbital and transcardially perfused with 30 ml heparinized saline (15 ml/min) followed by 200 ml fixative composed of 2% paraformaldehyde, 0.05% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB) (pH 7.4). The brains were removed from the skull and cryoprotected by overnight storage in 30% sucrose in 0.1 M PB. Thereafter, immunostaining was carried out on frozen sections coronally cut at a thickness of 20 μm. The tissue sections were preincubated for 15 min in 0.1% H_2O_2 in phosphate-buffered saline (PBS), subsequently rinsed in PBS, and immersed in 5% normal sheep serum (NSS) in PBS for 30 min to reduce aspecific binding in the following incubation step. Next, the sections were incubated with the first antibody [36G9, monoclonal mouse anti-PKCγ IgG raised against purified bovine PKCγ (Cazaubon et al., 1989, 1990)] diluted 1:200 in 1% NSS in PBS overnight at 4°C under gentle movement of the incubation medium. After the primary incubation, sections were rinsed in PBS and again preincubated with 5% NSS for 30 min before the secondary incubation step in biotinylated sheep anti-mouse IgG (Amersham), diluted 1:200 in PBS for 2 hr at room temperature (RT). Thereafter, the sections were thoroughly rinsed in PBS and incubated in streptavidin-HRP (Zymed) diluted 1:200 in PBS for 2 hr at RT. Finally, after subsequent rinsing in PBS and Tris buffer, the sections were processed by the diaminobenzidine (DAB)–H_2O_2 reaction (30 mg DAB and 0.01% H_2O_2/100 ml Tris buffer), guided by a visual check. Control experiments were performed by the omission of the primary antibody (36G9), yielding immunonegative results.

Table 1. Behaviorally induced changes in PKCγ-ir in mouse hippocampus compared with group N animals

Animals	CA1 pyramidal cells	CA3–CA4 pyramidal cells	DG granule cells	DG interneurons
H group	+	+	+	0
PT group	±	±	±	+
T group	++	++*	++	+

This table presents the summarized, semiquantified changes in the level of 36G9-ir as compared with the characteristics of the low-to-moderate level of 36G9-ir in N group mice. In group N mice, a relatively homogeneous distribution throughout the entire hippocampal formation was observed. Selective changes were observed in the H, PT, and T group animals. Symbols for staining intensity of cell bodies and dendrites: 0, minor changes; +, increase in cell bodies; ±, increase in dendrites; ++, strong increase in cell bodies and dendrites.

* Most notably in CA3c–CA4.

The 36G9 immunoreactivity (ir) of the H, PT, and T group mice was compared with the 36G9-ir of the N group mice. The changes in 36G9-ir for the H, PT, and T groups were semiquantified and presented in Table 1. Photomicrographs taken from the DAB-processed material were taken under identical exposure conditions and all coprocessed under similar printing conditions in the darkroom.

Phorbol ester binding to fixed brain sections. Shortly fixed cryostat sections of group N animals containing the hippocampus were preincubated for 2 hr at RT with a 100 μM concentration of the potent phorbol ester phorbol 12,13-dibutyrate (PDBu; Sigma). Subsequently, the staining procedure for 36G9 was performed as described above in the presence of 100 μM PDBu during the primary antibody incubation step. In parallel, two control experiments were performed on adjacent sections: (1) 36G9 incubation without PDBu treatment and (2) PDBu treatment as described above in the absence of 36G9 during the first incubation step to determine the possible binding of the secondary antibody and/or streptavidin-HRP to the bound PDBu.

Results

Spatial orientation in the hole board

The PT group mice crossed the hole board without performing a consistent search strategy. Instead, they randomly visited the holes in the absence of clear hole preferences. In contrast, the T group animals gradually learned to orientate in the hole board and to distinguish food holes from nonfood holes. Their averaged daily RMR, increasing from 0.27 (± 0.06 SEM) at day 6 (first day of testing) to 0.74 (± 0.12 SEM) at day 12, is shown in Figure 1. This increase in RMR displayed a statistical significance in time as compared with day 6 on days 7, 10, 11, 12, and 13, showing improvement in hole board performance during the experiment. No further increase in RMR was observed on the last day of testing (day 13; RMR = 0.59), indicating that the animals did not reach the maximal reference memory index representing a performance without errors. Twenty-four hours after the last trial, the animals were killed and hippocampal brain sections stained for PKCγ employing the monoclonal antibody 36G9.

36G9-ir in the hippocampus of group N mice

Application of 36G9 to tissue sections from group N mice revealed a characteristic pattern of low-to-moderate levels of PKCγ-ir in granule, pyramidal, and nonpyramidal cells

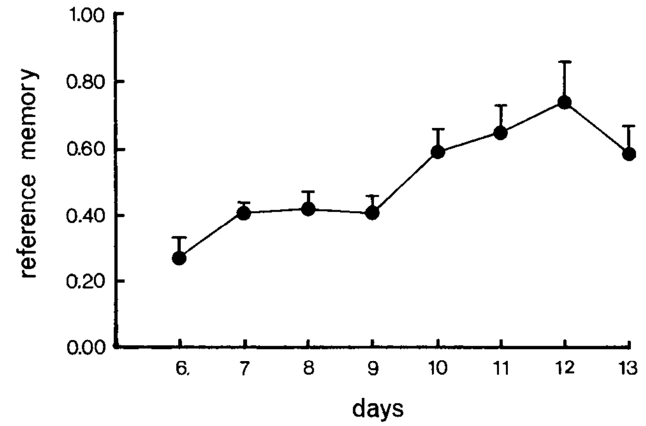

Figure 1. Mean reference memory performance per day of the T group mice ($n = 8$) on 8 consecutive days in the holeboard. The animals show a gradually increasing level of spatial orientation. Statistical significance in improvement of the RMR as compared with day 6 is found on days 7 ($p < 0.05$), 10 ($p < 0.01$), 11 ($p < 0.001$), 12 ($p < 0.001$), and 13 ($p < 0.01$).

throughout the entire hippocampus (Figs. 2*A,E*; 3*A,C*). No apparent differences were found between the dorsal, posterior, and ventral parts of the hippocampus. The pyramidal neurons of cornu Ammonis field CA1 displayed low levels of immunoreactivity (Figs. 2*A*, 3*A*), whereas pyramidal cells in CA3c–CA4 were stained somewhat stronger. The apical dendrites of the pyramidal cells were only faintly immunoreactive (Figs. 2*A*, 3*A*). The granule cells of the dentate gyrus (DG) revealed low levels of immunostaining (Figs. 2*E*, 3*C*). Nearly all interneurons of the stratum radiatum appeared to be immunonegative, and only a small portion of the interneurons of the stratum oriens were found positive. However, the interneurons of the polymorphic layer of the DG exhibited the strongest labeling of all hippocampal cell types, with clear dendritic profiles present in the surrounding tissue (Fig. 2*E*). In all immunopositive neurons, the cell membrane revealed the highest immunoreactivity whereas the cytoplasm displayed a considerably lower staining intensity.

36G9-ir in the hippocampus of group H and PT mice

The pattern of 36G9-ir in mice of the H and PT groups exhibited clear differences with the 36G9-ir observed in the group N mice (see Table 1; Fig. 2*B,F* and *C,G*). In most of the group H animals, the cell bodies of all immunoreactive neurons throughout the entire hippocampus appeared to be stronger stained (Fig. 2*B,F*). Such a clear increase was not observed in the group PT mice (Fig. 2*C,G*). However, the labeling intensity of the pyramidal apical dendrites and the dendrites of the dentate granule cells was selectively increased in the group PT animals. As an exception, in two animals of the H group small areas of highly immunoreactive pyramidal and granule cells were observed. In two PT group animals, small areas of strongly enhanced 36G9-ir in pyramidal cell bodies and dendrites were found. These findings appeared to be characteristic for all T group animals, which will be described below. In addition to these small changes in the hippocampal principal neurons, a striking increase in labeling intensity was seen in the interneurons of the polymorphic layer of the DG in the PT group (Fig. 2*G*). This change was not observed in the group H animals. The semiquantified differences between the N, H, and PT groups are summarized in Table 1.

36G9-ir in the hippocampus of group T mice

In contrast to the H and PT group mice, prominent changes in PKCγ staining became apparent in all T group mice. Large areas of CA1 and, to a somewhat lesser extent, CA3–CA4 (most notably CA3c–CA4) displayed a dense increase in 36G9-ir. The changes in staining intensity were most prominent in the pyramidal cell bodies, in their heavily branching apical dendrites in the stratum lacunosum/moleculare (Figs. 2*D*, 3*B*), and in the dentate granule cells (Figs. 2*H*, 3*D*). The observed increase in 36G9-ir in granule cells was predominantly found in the cell bodies, and was less dramatic in the dendrites. No further increase in staining intensity of the interneurons in the polymorphic layer of the DG or other hippocampal regions was found as compared with the PT group mice. The semiquantified changes displayed by the T group induced by hole board learning are summarized in Table 1.

36G9-ir after in vitro *manipulation of PKC by cofactor binding*

Since the epitope of 36G9 appeared to be affected by PKC cofactor binding such as diacylglycerol-analogs (DAG-analogs; Cazaubon et al., 1989), we examined whether phorbol ester binding to PKCγ changed the cellular 36G9-ir. Therefore, we pretreated mildly fixed brain sections of group N mice with 100 μM of the PKC activator (and DAG analog) PDBu. Under these conditions, the 36G9-ir dramatically increased (Fig. 4). This effect was observed in all brain areas containing PKCγ-positive neurons. Drastic changes were observed in the pyramidal and granule cells of the hippocampus (Fig. 4*B,D*). Some nonpyramidal neurons, notably in the strata radiatum and lacunosum-moleculare, also revealed pronounced 36G9-ir (arrows in Fig. 4*B*). After PDBu binding, especially the immunoreactivity of the pyramidal apical dendrites and the cell membrane of the principal cells increased, while no apparent increase in cytoplasm labeling was observed. Omission of the primary antibody 36G9 in the control sections treated with PDBu during the staining procedure resulted in total absence of immunoreactivity.

Discussion

The present study shows changes and redistribution of PKCγ-ir in hippocampal principal cells induced by spatial orientation in a hole board. A shift in 36G9-ir appeared from the cell somata to the dendrites when the animals of groups H and PT were compared. However, animals of the T group revealed strong PKCγ-ir in both cell somata and dendrites, which clearly exceeded that of the H and PT animals.

Changes in 36G9-ir after in vitro *manipulation of PKC by cofactor binding*

The present results show that the intensity of cellular immunostaining of PKCγ with antibody 36G9 is enhanced after prolonged phorbol ester treatment. After PDBu binding on cryosections, increased 36G9 staining was predominantly localized along the plasma membrane of the perikarya and dendrites, suggesting a selective change in membrane-bound PKCγ. This finding is in agreement with that of Olds et al. (1989), who reported that PDBu selectively binds to membrane-associated PKC. Phospholipids, necessary for PDBu binding to PKC, are absent in the cryosectioned cytoplasm of brain sections as cur-

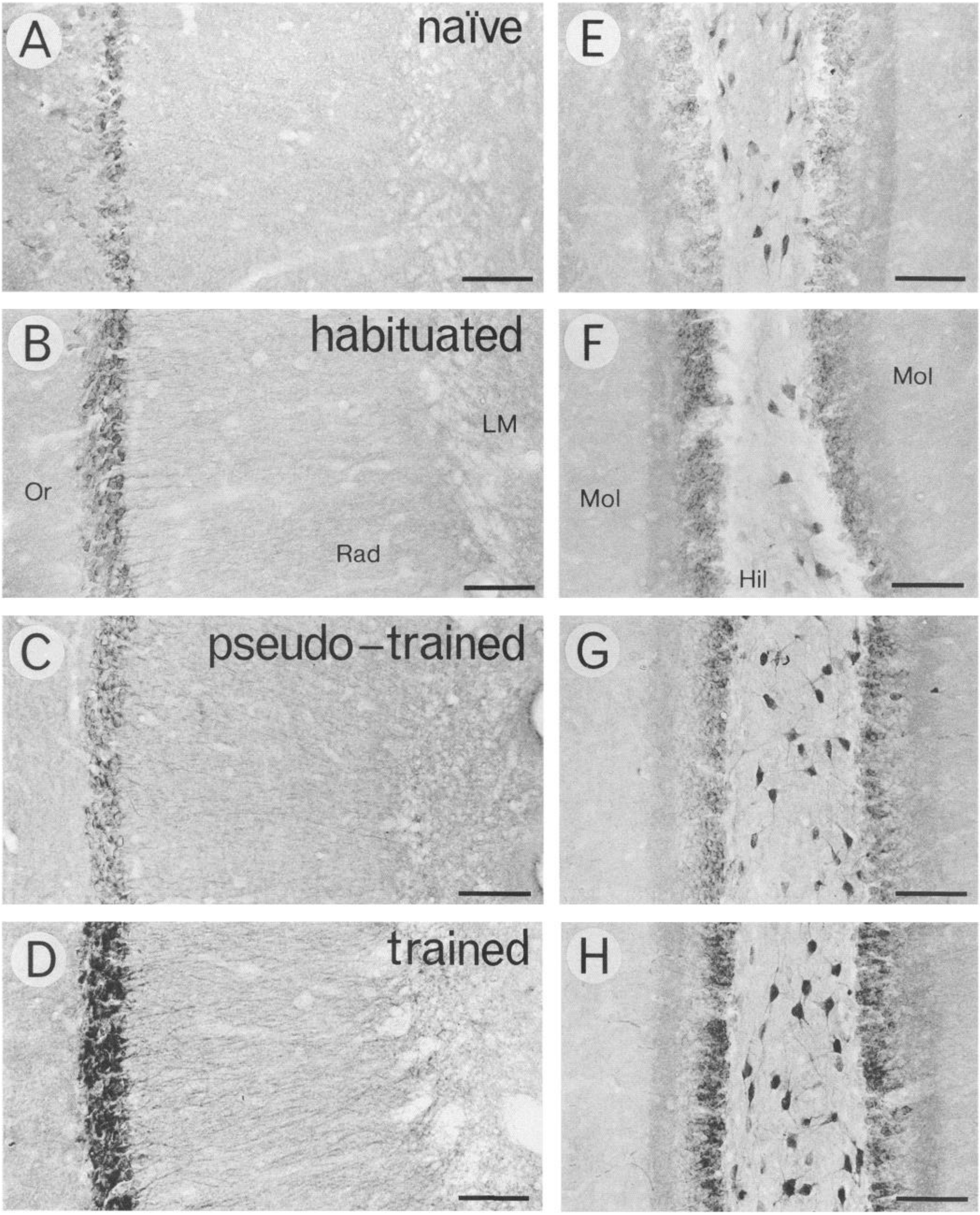

Figure 2. Low-power photomicrographs of the PKCγ labeling in CA1 and DG regions of all groups used. An increase in staining intensity of the cell bodies is observed in the group H (*B, F*) and group T (*D, H*) animals as compared with the group N animals (*A, E*). In the group PT animals (*C, G*), the PKCγ-ir in the cell bodies is decreased again. In contrast, a steady shift in enhanced PKCγ-ir from the group N to the group T animals is found for the apical dendrites of the pyramidal neurons (*A–D*) and, to a lesser extent, for the dendrites of the granule cells (*E–H*). The interneurons in the DG revealed an identical shift in labeling intensity (*E–H*). *Hil*, hilus; *LM*, stratum lacunosum/moleculare; *Mol*, stratum moleculare; *Or*, stratum oriens; *Rad*, stratum radiatum. Scale bars, 70 μm.

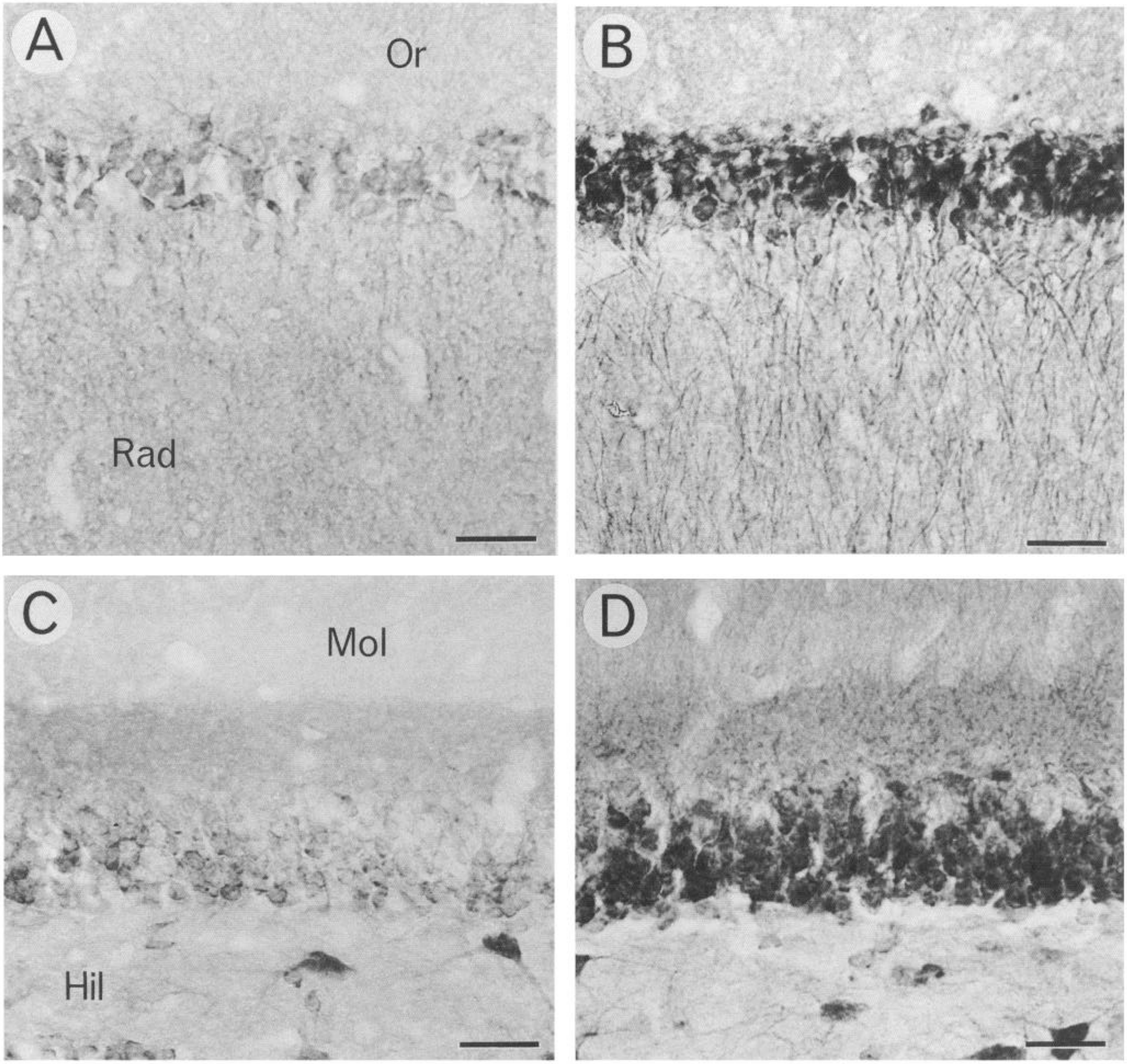

Figure 3. High-power photomicrographs depicting the dramatic increase in PKCγ labeling as observed in the group T mice (*B, D*) compared with group N mice (*A, C*). The increase is evident in both pyramidal neurons and their apical dendrites (*A, B*) and granule cells and associated dendrites (*C, D*). *Hil*, hilus; *Mol*, stratum moleculare; *Or*, stratum oriens; *Rad*, stratum radiatum. Scale bars, 25 μm.

rently used to study the effect of PDBu treatment on 36G9-ir (Olds et al., 1989).

The observed increase in 36G9-ir after phorbol ester binding to fixed brain sections is caused neither by 36G9 cross-reactivity to bound phorbol ester nor by aspecific binding of the secondary antibody to the bound PDBu. First, Cazaubon et al. (1990) demonstrated that 36G9 does not bind to PDBu or interact with the binding site for phorbol ester. Second, control experiments in which 36G9 was omitted from the incubation medium revealed that an aspecific interaction of bound PDBu with the secondary antibody did not occur. The increase in 36G9-ir after phorbol ester binding mimics the observed increase in 36G9-ir of the hippocampal principal cells after hole board learning, indicating a modulation of PKCγ function. Likewise, short-term treatment (20 min) of fresh brain slices (500 μm thickness) with 100 μM of the cholinergic agonist carbachol (as performed by Olds et al., 1989) caused a strong increase in 36G9-ir (E. A. Van der Zee, unpublished observations). The observed increase in 36G9-ir after carbachol treatment shows that 36G9-ir, in addition to direct PKC manipulation by phorbol esters, can be changed after indirect stimulation of PKCγ through receptor stimulation. These results may not be surprising, since conformational change(s) in the PKCγ molecule results in changed 36G9 binding to purified PKCγ (Cazaubon et al., 1989). Furthermore, the epitope recognized by 36G9 has been shown to be functionally related to the phorbol ester binding site on the purified PKCγ molecule (Cazaubon et al., 1989, 1990).

In *in vivo* conditions, after receptor activation and subsequent stimulation of phospholipase C, DAG and inositol trisphosphate (ip3) are produced. Translocation of PKCγ from the cy-

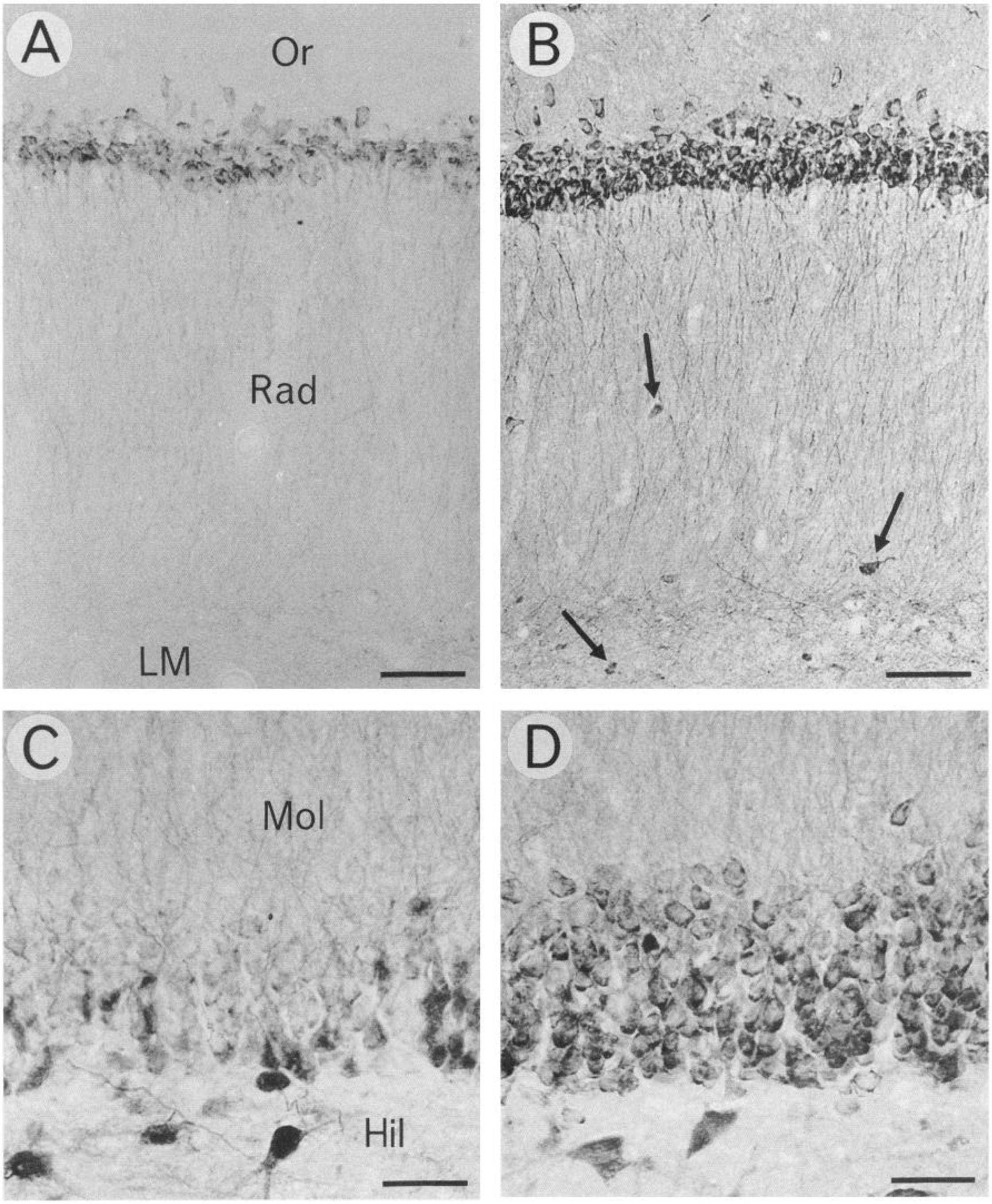

Figure 4. Photomicrographs showing the prominent increase in 36G9-ir after phorbol ester (PDBu) treatment of fixed brain sections. PDBu treatment on hippocampal sections of group N mice (*B, D*) revealed a clear increase in 36G9-ir compared with adjacent nontreated sections (*A, C*). The characteristics of the increase strongly resemble those depicted in Figure 3, showing the learning-induced increase in hippocampal PKCγ-ir. In contrast to the behaviorally induced increase, PDBu treatment revealed an additional increase in 36G9-ir in the nonpyramidal neurons of strata radiatum and lacunosum/moleculare (*arrows* in *B*). *Hil*, hilus; *LM*, stratum lacunosum/moleculare; *Mol*, stratum moleculare; *Or*, stratum oriens; *Rad*, stratum radiatum. Scale bars: *A* and *B*, 40 μm; *C* and *D*, 20 μm.

tosol to the membrane through DAG binding in combination with other cofactors (e.g., calcium and phospholipids) activates PKCγ. Enhanced 36G9-ir most probably reflects increased excitatory synaptic transmission upon cells by which PKCγ functioning is altered. In this way enhanced 36G9-ir identifies neurons utilizing PKCγ in brain regions involved in learning and memory processes.

Behaviorally induced changes in 36G9-ir

Enhanced hippocampal 36G9-ir suggests, as discussed above, an increase in hippocampal synaptic activity. Comparing the N, H, and PT groups, a shift in enhanced 36G9-ir appears from the cell somata during the first days of habituation (represented by the H group animals) to the dendrites (most notably in the pyramidal cells) after prolonged habituation (represented by the PT group animals). These results suggest that synaptic transmission, by which 36G9-ir is enhanced, is shifted from the cell body layer to the dendrites. A possible additional explanation for the enhanced 36G9-ir is *de novo* synthesis of PKCγ. The balance between PKCγ synthesis and degradation might be changed, by which the total pool of PKCγ is enlarged. Newly synthesized PKCγ might be time-dependently transported from the cell somata to the dendrites. Similarly, Olds et al. (1989) suggested redistribution of PKC within rabbit CA1 pyramidal cells from the cell somata 24 hr after conditioning to dendrites 72 hr after conditioning due to *de novo* synthesis of PKC.

In contrast, the T group mice displayed strong 36G9-ir in *both* cell somata and dendrites, which clearly exceeded that of the H and PT group mice. This may be explained by an ongoing production of PKCγ and/or a continuing synaptic activity upon both cell bodies and dendrites as a consequence of the acquisition of the learning task. One can speculate that the PKC shift from cell somata to dendrites is not yet fulfilled in the T group mice, since the behavioral data showed that they had not yet reached the learning asymptote and hence were killed at a time of behavioral acquisition.

Besides learning-induced changes in 36G9-ir, a more general and aspecific activation of the hippocampus may have contributed to the enhanced 36G9-ir in the H, PT, and T group animals. Neuronal plasticity of hippocampal pyramidal cells can be induced by handling itself (Horner et al., 1991), an integral part of the behavioral paradigm of the H, PT, and T groups. In addition to the aspecific hippocampal activation, one should realize that during the habituation of group H and, even more pronounced, during the prolonged habituation of the PT group, acquisition of working memory through hippocampal activation may have started (Van der Staay et al., 1990).

The changes in PKCγ-ir notably in the T group were most prominent in the pyramidal cells of CA1 and granule cells of the DG. In addition, parts of the neocortex exhibited clear increases in 36G9-ir. Such changes appeared to be specific to spatial discrimination, since hole board experience without a discrimination between baited and nonbaited holes did not cause consistent changes in cortical 36G9-ir. Neocortical changes related to learning are probably caused by consolidation processes, since it has been proposed that the hippocampal formation has a temporary role in memory, whereas a more permanent memory resides within the neocortex (Zola-Morgan and Squire, 1990).

Possible neurotransmitters involved in the activation of PKCγ induced by hole board learning

The activation of PKCγ induced by spatial learning observed in the present study can be mediated by different receptor types linked to phosphatidylinositol (PI) turnover. These receptors are stimulated by specific neuronal pathways involved in learning and memory processes. Two likely candidates for the activation of PKC are acetylcholine (ACh) and glutamate. The cholinergic septohippocampal projection (Clarke, 1985; Nyakas et al., 1986) activates PKC through its action upon muscarinic ACh receptors (mAChRs) (Fisher and Bartus, 1985). PKCγ appeared to be highly expressed in neurons possessing mAChRs (Van der Zee et al., 1990). Currently, we are examining changes in mAChR characteristics induced by spatial discrimination learning in mouse hippocampus by means of the anti-mAChR antibody M35 (Van der Zee et al., 1989, 1991). The glutamatergic entorhinal projection to the hippocampus terminates on the apical dendrites of the pyramidal and granule cells (Doller and Weight, 1982; Witter et al., 1988). Glutamate excites hippocampal pyramidal cells (Hvalby, 1990), and some of the hippocampal glutamate receptors are linked to PI turnover (Hwang et al., 1990; Stratton et al., 1990). However, most probably both cholinergic and glutamatergic stimulation contribute to the changes in 36G9-ir induced by spatial navigation in the hole board.

The present results, showing changes in hippocampal PKCγ by means of 36G9-ir induced by hole board learning, are consistent with the observation that PKC activation is necessary for proper spatial memory performance (Wehner et al., 1990; Paylor et al., 1991). Neuronal plasticity as visualized by 36G9-ir leads to additional anatomical information in relation to learning and memory processes as compared with results obtained with tritiated phorbol esters (Bank et al., 1988; Olds et al., 1989, 1990; Olton et al., 1991; Scharenberg et al., 1991), or autoradiographically imaged PI turnover (Hwang et al., 1990). Therefore, changes in 36G9-ir shed more light on the contribution of (hippocampal) neurons using PKCγ in the acquisition of spatial orientation as well as other types of learning tasks (Luiten et al., 1991).

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