Exposure to Chronic Psychosocial Stress and Corticosterone in the Rat: Effects on Spatial Discrimination Learning and Hippocampal Protein Kinase Cγ Immunoreactivity

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ABSTRACT: Previous reports have demonstrated a striking increase of the immunoreactivity of the γ-isof orm of protein kinase C (PKCγ-ir) in Ammon's horn and dentate gyrus (DG) of rodent hippocampus after training in a spatial orientation task. In the present study, we investigated how 8 days of psychosocial stress affects spatial discrimination learning in a hole board and influences PKCγ-ir in the hippocampal formation.

The acquisition of both reference memory and working memory was significantly delayed in the stressed animals during the entire training period. With respect to cellular plasticity, the training experience in both nonstressed and stressed groups yielded enhanced PKCγ-ir in the CA1 and CA3 regions of the posterior hippocampus but not in subfields of the anterior hippocampus. Stress enhanced PKCγ-ir in the DG and CA3 pyramidal cells of the anterior hippocampus. In stressed animals that were subsequently trained, the PKCγ-ir was increased in the posterior CA1 region to the same level as that found in nonstressed trained animals. Stress apparently abrogated the PKCγ-ir training response in the CA3 region.

In a second experiment, the elevation of plasma corticosterone levels to values that are found during stress did not significantly influence reference memory scores but slightly and temporarily affected working memory. The training-induced enhancement of PKCγ-ir in the CA1 region was similar in trained and corticosterone-treated trained animals, but the learning-induced PKCγ-ir response in the posterior CA3 area was absent after corticosterone pretreatment. These results reveal that prolonged psychosocial stress causes spatial learning deficits, whereas artificial elevation of corticosterone levels to the levels that occur during stress only mildly affects spatial memory performance. The spatial learning deficits following stress are reflected only in part in the redistribution of hippocampal PKCγ-ir following training. Hippocampus 7:427–436, 1997.

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KEY WORDS: learning and memory; spatial orientation; stress; corticosterone; hippocampus; protein kinase C; dentate gyrus

INTRODUCTION

The experience of stress triggers a complex neuroendocrine cascade that leads to, among other effects, the release of glucocorticoids from the adrenal gland. Both stress and glucocorticoids exert striking influences on cognitive behaviors. Brief exposure to stress can enhance the acquisition of a classically conditioned eyeblink response (Shors et al., 1992; Shors and Servatius, 1995), whereas long-term stress can impair spatial discrimination learning in rats (Luine et al., 1994; Bodnoff et al., 1995). Long-term exposure of rats to high doses of corticosterone attenuates cognitive performance (Dachir et al., 1993; Luine et al., 1993; Arbel et al., 1994; Bodnoff et al., 1995), whereas removal of the adrenal gland and replacement of appropriate levels of corticosterone prevents stress-induced cognitive deficits (Bodnoff et al., 1995). Stress and stress-induced levels of corticosterone also impair long-term potentiation and primed burst potentiation in the hippocampus, two phenomena that may represent cellular changes underlying mechanisms of learning and memory (Foy et al., 1987; Shors et al., 1989; Bennett et al., 1991; Diamond and Rose, 1990; Diamond et al., 1992, 1994; Pavlides et al., 1993, 1995a,b; Kerr et al., 1994).

Several studies on the cellular and molecular basis of learning and memory point to a role for protein kinase C (PKC) in memory processing. PKC, a phospholipid and Ca2+-dependent kinase, is a key enzyme in signal transduction and neuronal plasticity. Changes in the distribution of PKC in the hippocampus, which has
been implicated in many studies as a brain region pivotal to associative and spatial cognitive processes (O'Keefe and Nadel, 1978; M.C. Naughton et al., 1986; Schmajuk, 1990; Nadel 1991; Zola-Morgan and Squire, 1990; O'Mara, 1995), have been associated with several forms of associative learning (Olids et al., 1989, 1990; Scharenberg et al., 1991; Beldhuis et al., 1992; Van der Zee et al., 1992, 1995a; Sunayashiki-Kusuzaki et al., 1993). In addition, PKC inhibitors and PKC activators such as phorbol esters alter memory performance (Paylor et al., 1991; Zhao et al., 1994; Nogues et al., 1996), and mouse hippocampal PKC activity correlates positively with the ability to learn a spatial discrimination task (Wehner et al., 1990). Of the different isoforms of PKC present in the brain, the γ-subtype is the most abundant representative in the rat hippocampus (Huang et al., 1988; Saito et al., 1988) and is associated with spatial learning performance (Beldhuis et al., 1992; Van der Zee et al., 1992, 1995a; Abeliovich et al., 1993). The studies by Van der Zee et al. (1992) and Beldhuis et al. (1992b) have revealed a striking increase of the immunoreactivity of the γ-isozyme of protein kinase C (PKC-γ-ir) in the pyramidal cell layer of the Cornu Ammonis 1 (CA1) and dentate gyrus (DG) of mouse and rat hippocampus after training in a spatial orientation task.

The first aim of the present study was to investigate the impact of prolonged stress on reference and working memory. Whereas noxious stimuli or severe physical stressors have been used in most studies investigating the effects of stress on memory, the most naturally occurring stress is of psychological origin. Such stress can have a profound impact on the behavior, neurobiology, and physiology of rodents (Blanchard et al., 1993; Krugers et al., 1996). For that reason, we investigated the effect of psychosocial stress resulting in submission to a dominant male rat (Koolhaas et al., 1990; Krugers et al., 1996). The confrontation took place in a wooden cage (85 × 60 × 50 cm) that was permanently occupied by the dominant rat. The social interaction resulted by definition in the display of submissive behavior by the experimental animal. After submission, the experimental rat was put into a small nestbox in the home cage of the dominant rat for the next 8 days. The small nestbox was inaccessible to the dominant rat. In a pilot study, the impact of this social stress on plasma corticosteroid levels during the 8-day stress experience was investigated. Thus, 7 animals were provided with a permanent silicon catheter (0.95 mm outer diameter, 0.50 mm inner diameter) in the right atrium inserted via the right jugular vein (Steffens, 1969). The animals were allowed to recover from surgery for 1 week. Subsequently, 2 animals were housed individually (controls) and 5 animals were socially stressed. Blood samples of 0.45 ml were taken daily 1 h after lights were switched off. Immediately after withdrawal, the blood samples were transferred to chilled (0°C) centrifuge tubes containing 10 μl heparin (500 IU/ml), centrifuged for 20 min at 3,500 g, and stored at −20°C until further processing. Corticosterone was extracted from 75 μl plasma by using a liquid extraction method (Shimizu et al., 1983). Quantification of plasma corticosterone was performed by high-pressure liquid chromatography in combination with ultraviolet detection. The absolute detection threshold for corticosterone in plasma was 8 ng/ml.

In the second experiment, we investigated the effect of corticosterone pellet implantation on spatial orientation in the hole board. Corticosterone (4-pregnene-11β,21-diol-3,20-dione; Sigma, St. Louis, MO) pellets were formed by slowly heating corticosterone and cholesterol (5-cholestene-3β-ol; Sigma) at a 1:1 ratio. The 50% corticosterone pellets weighed approximately 100 mg each (see Meyer et al., 1979). The animals were anesthetized with ether, and the pellets were implanted subcutaneously in the abdominal cavity at least 2 cm caudal to the incision. Whether these pellets produced transiently elevated circulating plasma corticosterone levels as observed during the 8 days of psychosocial stress was checked by a small control experiment. This experiment revealed that implantation of a 50% corticosterone pellet resulted in plasma corticosteroid levels of 25.5 ± 4.3 μg/dl on the first day after implantation which then slowly decreased to 11.7 ± 1.7 μg/dl by day 8 (unpublished observations). Cholesterol pellets (100 mg) served as controls. Naive (N) animals were kept in groups and served as blank controls, and animals from the training (T) group received a cholesterol pellet.

### Materials and Methods

#### Animals

Four-month-old male Wistar rats, bred in our own facilities, were housed in groups of 6–7 animals per cage and kept on a 12-h lights-on/12-h lights-off cycle (7:00–19:00 dark). Food and water were available ad libitum. The experimental protocols were approved by the Committee on Animal Bio-Ethics of the University of Groningen.

#### Stress and Corticosterone Treatment

Animals were exposed to 8 days of prolonged subordination stress by confrontation with a selected dominant male TMD S3 rat (Koolhaas et al., 1990; Krugers et al., 1996). The confrontation took place in a wooden cage (85 × 60 × 50 cm) that was permanently occupied by the dominant rat. The social interaction resulted by definition in the display of submissive behavior by the experimental animal. After submission, the experimental rat was put into a small nestbox in the home cage of the dominant rat for the next 8 days. The small nestbox was inaccessible to the dominant rat. In a pilot study, the impact of this social stress on plasma corticosteroid levels during the 8-day stress experience was investigated. Thus, 7 animals were provided with a permanent silicon catheter (0.95 mm outer diameter, 0.50 mm inner diameter) in the right atrium inserted via the right jugular vein (Steffens, 1969). The animals were allowed to recover from surgery for 1 week. Subsequently, 2 animals were housed individually (controls) and 5 animals were socially stressed. Blood samples of 0.45 ml were taken daily 1 h after lights were switched off. Immediately after withdrawal, the blood samples were transferred to chilled (0°C) centrifuge tubes containing 10 μl heparin (500 IU/ml), centrifuged for 20 min at 3,500 g, and stored at −20°C until further processing. Corticosterone was extracted from 75 μl plasma by using a liquid extraction method (Shimizu et al., 1983). Quantification of plasma corticosterone was performed by high-pressure liquid chromatography in combination with ultraviolet detection. The absolute detection threshold for corticosterone in plasma was 8 ng/ml.

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#### Spatial Orientation Task

The hole board task was used for spatial orientation learning (Oades and Isaacson, 1987; Beldhuis et al., 1992; Van der Zee et al., 1992). The setup consisted of a groundfloor plate of 70 × 70 cm, with four rows of four equidistant holes (14 cm apart, 3.5 cm in diameter, 3 cm deep). Animals were habituated to the procedure by introducing them to the hole board; the animals then spent two trials of 3 min each on 5 consecutive days in the test apparatus with food in all holes. The habituation period was followed by a training period of 10 daily sessions. During these
The animals were trained to find 4 holes that were filled with an accessible food pellet arranged in a fixed symmetrical pattern. Each hole contained a food pellet at the bottom and was covered by a replaceable, perforated false bottom. In this way, the rats were unable to discriminate between baited and nonbaited holes by olfactory stimuli. The animals were trained during two trials per day; each trial was 3 min. At the start of each trial, the animals were placed in a start box attached to one of the walls of the hole board. After 10 s, a guillotine door between the start box and the arena of the hole board was lifted, allowing the rat to enter the arena. A visit was scored when the nose of the rat was placed in a hole. Revisits to baited holes and visits to nonbaited holes were recorded as errors. The animals were removed from the hole board either after all 4 holes were visited or after the total testing time.

Between trials, the floors of the start box and hole board were cleaned with a wet and dry cloth. Reference memory was defined as the number of visits and revisits to the baited holes divided by the total number of visits to baited and nonbaited holes. Working memory was calculated as the ratio of the number of food rewarded visits to the number of visits and revisits to the baited set of holes (Beldhuis et al., 1992; Van der Zee et al., 1992).

**Stress, Corticosterone, and Spatial Orientation**

The animals were divided into four groups to establish the effect of 8 days of social stress on spatial learning in the hole board and PKCγ-ir levels in the hippocampus: naive (N, n = 5), trained (T, n = 7), stressed (S, n = 6), and stressed/trained animals (ST, n = 6). The N group was group-housed and served as controls; the S group was exposed to the 8 days of prolonged subordination stress. The T group was habituated and subsequently trained in the hole board. The ST group was subject to the subordination stress and underwent the habituation and training procedure after the stress exposure, as described for the T group.

To investigate whether the stress-induced alterations in spatial orientation and PKC could be attributed to the stress-evoked rise in plasma corticosterone levels, the animals were divided into four groups: naive (N, n = 6), trained (T, n = 7), corticosterone-treated (C, n = 5), and corticosterone-treated/trained (CT, n = 6) animals. Eight days after pellet implantation, the T group was habituated to the hole board for 5 days and subsequently trained for 10 days to find the 4 baited out of 16 holes. The C group received a corticosterone pellet. The CT group was supplied with a corticosterone pellet, and 8 days after surgery the animals underwent the habituation and training procedure, as described for the T group.

**Immunocytochemical Procedure**

Either 24 h after the last trial (for the animals trained in the hole board) after the last day of the stress procedure (S) or 9 days after corticosterone pellet implantation, the animals were sacrificed together with the naive animals. All rats were deeply anesthetized with ether and transcardially perfused with 30 ml heparinized saline (15 ml/min), followed by 300 ml fixative composed of 3% paraformaldehyde, 0.05% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (PB; pH 7.4). The adrenal and thymus glands were dissected and weighed.

The brains were removed from the skulls and cryoprotected by overnight storage in 30% sucrose in 0.1 M PB. Thereafter, 30-μm coronal sections were cut on a cryostat microtome, and sections were processed for detection of PKCγ. The immunocytochemical staining was performed on free-floating sections as described below; all steps being identical and run simultaneously for the animals in each experiment. The tissue sections were preincubated for 15 min in 0.1% H2O2 in phosphate buffered saline (PBS), rinsed in PBS, and immersed in 5% normal sheep serum (NSS) in PBS for 30 min to reduce background staining. Next, the sections were incubated with the first antibody (36G9, monoclonal mouse anti-PKCγ IgG raised against purified bovine PKC diluted to 1:1 in 0.01 M PBS; Cazaubon et al., 1989, 1990) overnight at 4°C under gentle movement of the incubation medium. After the primary antibody incubation, sections were rinsed in PBS and preincubated with 5% NSS for 30 min before exposure to biotinylated sheep anti-mouse IgG (Amersham; diluted 1:200 in PBS) for 2 h at room temperature (RT). Subsequently, the sections were thoroughly rinsed in PBS and incubated in streptavidin and horseradish peroxidase (Zymed; diluted 1:200) in PBS for 2 h at RT. After subsequent rinsing in PBS and Tris buffer, the sections were processed by the diaminobenzidine (DAB)–H2O2 reaction method (30 mg DAB and 0.01% H2O2/100 ml Tris buffer), guided by a visual check.

**Measuring Optical Density**

Sections of the animals of different groups were qualitatively analyzed, and the experimenters were unaware of the origin of the sample. In addition, the optical density (OD) of PKCγ-ir was measured with an image analysis system (IBAS) to obtain semiquantitative data on the distribution of PKCγ. The OD was measured in the CA1 and CA3 pyramidal cell layer and in the DG molecular and granular cell layer at anterior (I.A. 5.7, according to Paxinos and Watson, 1982) and posterior (I.A. 4.2) levels. The OD values of the corpus callosum served as a measure for nonspecific background staining. Specific staining was calculated by subtraction of the OD of the background from the total OD. Four hippocampi per animal per level were analyzed. Data per group were averaged and compared for relative differences between groups.

**Statistics**

All data were averaged and calculated as mean ± SEM per group. The reference memory and working memory scores were statistically analyzed with multivariate analysis of variance (MANOVA) for repeated measures by using stress as the between-subjects factor and daily training session as the within-subjects factor, followed by post hoc analysis. The OD of the different groups was compared with the OD of the N group and statistically analyzed by MANOVA. Significance was considered to be P < 0.05.
RESULTS

Body Weight, Thymus and Adrenal Weights, and Plasma Corticosteroid Levels

Stress experience

Changes in body weight during the 8 days of social stress are presented in Figure 1A. Stressed rats lost up to 7% of their body weight during the first 3 days of the stress experience. From day 4, the stressed animals gained again body weight, but their absolute weight remained lower when compared with nonstressed animals. Statistical analysis revealed a significant effect of stress on body weight \[F(1,9) = 23.98, P < 0.001\]. The social stress regime had also a profound effect on adrenal and thymus weight (Table 1): it reduced relative thymus weight and increased relative adrenal weight. No significant stress effects on adrenal or thymus weights were observed in T and ST rats.

The pilot experiment revealed that rats subjected to 8 days of subordination stress also underwent significant changes in plasma corticosteroid levels (Fig. 1B). During the first 2 days, plasma corticosteroid levels of stressed animals were significantly elevated but then gradually declined to control levels.

![Figure 1A](image.png)

![Figure 1B](image.png)

**FIGURE 1.** The effect of chronic stress on (A) body weight gain and (B) plasma corticosteroid levels (\(\mu g/100\) ml). Values represent the mean ± SEM. Asterisks represent significant differences from the control group \((P < 0.05)\).

**FIGURE 2.** Changes in body weight during 8 days of rats treated with corticosterone vs. control rats. Body weight was significantly reduced over the entire period in the stressed animals when compared with control rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Thymus weight (mg/100 g body weight)</th>
<th>Adrenal weight (mg/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>5</td>
<td>13.98 ± 1.00</td>
<td>5.24 ± 0.59</td>
</tr>
<tr>
<td>Stress</td>
<td>6</td>
<td>5.95 ± 0.96*</td>
<td>8.45 ± 1.22*</td>
</tr>
<tr>
<td>Training</td>
<td>7</td>
<td>13.07 ± 0.58</td>
<td>4.92 ± 0.53</td>
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<tr>
<td>Stress/training</td>
<td>6</td>
<td>10.34 ± 1.22</td>
<td>6.00 ± 0.60</td>
</tr>
</tbody>
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*Values represent the mean ± SEM. Asterisks indicate significant differences from naive group.

Corticosterone treatment

Rats implanted with a cholesterol pellet showed little weight loss after the surgical procedure; within 6 days, their weight gain reached the preoperative value (Fig. 2). In contrast, rats implanted with a corticosterone pellet lost about 5% of their body weight in the first 2 days after the implantation, and it took at least 11 days to regain this loss. Statistical analysis by MANOVA showed a significant difference between the groups over the 8-day period \([F(2,6) = 6.37, P < 0.001]\). The corticosterone pellet implantation reduced thymus weight, whereas adrenal weight was not significantly altered (Table 2). No effect of corticosterone on thymus weight was observed after subsequent training of the animals.

Spatial Orientation in the Hole Board

Stress experience

Development of reference memory ratio (RM) and working memory ratio (WM) during training for T and ST animals is presented in Figure 3. The between-groups comparison indicated

<table>
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TABLE 2. Correlates of Stress on Thymus and Adrenal Weight Corrected for Body Weight

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</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>10.02 ± 0.92</td>
<td>5.25 ± 0.43</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>7.55 ± 0.28*</td>
<td>4.44 ± 0.59</td>
</tr>
<tr>
<td>Training</td>
<td>8.93 ± 0.60</td>
<td>5.31 ± 0.20</td>
</tr>
<tr>
<td>Corticosterone/training</td>
<td>8.46 ± 0.79</td>
<td>4.98 ± 0.20</td>
</tr>
</tbody>
</table>

*Corticosterone treatment

The immunocytochemical distribution of PKCγ-ir in the hippocampus of N animals was similar to that described above. There were no changes in PKCγ-ir in the anterior hippocampus (Fig. 8). In the posterior hippocampus, however, training led to enhanced PKCγ-ir increase was particularly strong in anterior dentate granular cells and in their neurites in the dentate molecular layer. No such change was present at posterior CA3 and DG levels. These qualitative observations were substantiated by the semiquantitative measurement of the OD of PKCγ-ir (Figs. 5, 6). Training in the hole board did not affect PKCγ-ir in the anterior hippocampus, but training specifically enhanced PKCγ staining in the posterior hippocampus, most notably in the pyramidal cell bodies and their apical dendrites in the stratum radiatum of the CA1 and CA3 region (Figs. 5-7). Eight days of stress did not influence the training-enhanced PKCγ-ir in the posterior CA1 region, which was similar to the immunoreactivity in T animals without previous treatments (Fig. 7). In posterior CA3 neurons, the preceding stress inhibited the training-induced increase in PKCγ-ir (Figs. 5, 6).

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**FIGURE 3. Development of reference memory ratio (A) and working memory ratio (B) of trained (n = 7) and stressed-trained animals (n = 6). The between-groups measurements indicate significantly impaired RM (A) and WM (B) scores for stressed animals over the entire period (P < 0.05). Analysis of variance indicates significant differences in both RM and WM at several days, which are indicated by asterisks (P < 0.05).**
PKCγ-ir in large areas of the CA1 and CA3 region. These changes were most prominent in the pyramidal cell bodies and their apical dendrites in stratum radiatum (Fig. 9). Although the effect of training on the enhanced PKCγ-ir in posterior CA1 pyramidal cells was similar in T and CT animals, it was abrogated by corticosterone treatment in the posterior CA3 pyramidal region (Fig. 9).

DISCUSSION

First, these two experiments show that 8 days of psychosocial stress impairs both reference memory and working memory in a spatial orientation paradigm. Second, both hole board training and stress have an impact on the plasticity of PKCγ-ir in the rat hippocampus. PKCγ-ir in posterior hippocampal CA1 and CA3 pyramidal cells was enhanced by training, whereas stress specifically increased PKCγ-ir in DG and CA3 at anterior hippocampal levels. Remarkably, stress prior to spatial learning did not affect the training enhanced PKCγ-ir in the CA1 region of the hippocampus but appeared to abrogate learning-induced PKCγ changes in the CA3 region. Third, artificial elevation of plasma corticosterone levels to stress values did not affect reference memory but affected the early phase of development of working memory. With respect to cellular plasticity, the elevated corticosteroid levels did not affect the training-induced increase of PKCγ-ir in the CA1 pyramidal cells but prevented the training-enhanced PKCγ-ir in the CA3 pyramidal cell layer.

Stress and Memory

A couple of studies have demonstrated the effects of prolonged stress on memory performance. Luine et al. (1994) reported impaired memory performance in an 8-arm radial maze after 3 weeks of restraint stress, and Bodnoff et al. (1995) described learning deficits in a water maze after 6 months of social stress. In the present study, 8 days of psychosocial stress of submission to a dominant male rat were used to investigate the impact of stress on learning in a hole board spatial discrimination task. This stress paradigm caused an acute drop in body weight gain, transiently elevated basal corticosterone levels, and altered adrenal and thymus weights, thereby corroborating the stressful character of stress and memory.

FIGURE 4. Development of RM (A) and WM (B) of trained (n = 7) and corticosterone-treated trained animals (n = 6). The between-groups measurements did not reveal significant differences in RM (A) or in WM (B) over the entire period (P < 0.05).

FIGURE 5. Optical density (OD) of PKCγ-ir in the CA1 and CA3 pyramidal cell layers, dentate gyrus molecular layer (ML), and the inner (DG in) and outer (DG out) blades of the dentate gyrus granular cell layer in the anterior hippocampus (I.A. 5.7 according to Paxinos and Watson, 1982). Stress significantly increased PKCγ-ir in the CA3 pyramidal cell layer (A) and dentate gyrus granular and molecular layer (B) when compared with those of naive animals (*P < 0.05). Naive (n = 5), trained (n = 7), stressed (n = 6), and stressed/trained (n = 6) rats are shown. The insert at the top indicates the selected regions for measuring the OD.
the interaction between the experimental and dominant rat (De Goeij et al., 1992; Blanchard et al., 1993; Krugers et al., 1996). Our present results reinforce the notion that prolonged stress causes learning deficits and show that (a) social stress affects both reference and working memory and (b) a period of 8 days of social stress is sufficient to cause spatial learning deficits.

Corticosterone and Memory

Several indirect lines of evidence suggest that corticosteroids are involved in the stress-induced reduction of memory performance (Dachir et al., 1993; Arbel et al., 1994; McEwen and Sapolsky, 1995). Direct evidence that elevated levels of corticosterone may mediate spatial learning deficits after stress was presented by Bodnoff et al. (1995). They reported that middle-aged stressed animals, which were adrenalectomized and supplied with low levels of corticosterone, failed to display cognitive impairments. The 50% corticosterone pellets in our study transiently elevated basal corticosterone levels to values similar to those observed during the 8 days of social stress. However, this elevation had no effect on spatial discrimination learning. Reference memory scores were similar between T and CT animals, and although corticosterone treatment slowed down the working memory acquisition rate in the early phase of the training period, no significant differences over the entire period were observed. No cognitive effects of corticosterone were observed, possibly for the following reasons. First, corticosterone has only been reported to cause cognitive deficits when administered in supraphysiological dosages (Dachir et al., 1993; Arbel et al., 1994; Bodnoff et al., 1995) and during longer periods (Dachir et al., 1993; Luine et al., 1993; Arbel et al., 1994; Bodnoff et al., 1995) than in our study. Second, corticosterone appears to be more effective in attenuating spatial memory processes in middle-aged than in the young adult rats used in the present experiments (Arbel et al., 1994; Bodnoff et al., 1995). Third, corticosterone may be responsible for stress effects on cognition during the actual elevation of this hormone level, as has been suggested by a few studies (e.g., Oitzl and de Kloet, 1992; Sandi and Rose, 1994a,b). Thus, the long-lasting action of stress we observed in the present study seems to involve other stress-related mechanisms (Bohus, 1994).

Stress, Corticosterone, and Learning-Induced Alterations in PKC

In the present study, we observed an increase in hippocampal PKCγ-ir induced by spatial orientation in a hole board similar to
that reported previously using the same learning test (Beldhuis et al., 1992; Van der Zee et al., 1992, 1995a). Redistribution and changes in PKC after learning are consistent with the observation that activation of PKC is necessary for proper spatial memory performance (Wehner et al., 1990; Paylor et al., 1991). The results on training-enhanced PKC-ir are also in agreement with the reported changes in the intracellular distribution of PKC in the CA1 and CA3 region, which accompany associative memory storage within the hippocampus (Olds et al., 1989, 1990; Scharenberg et al., 1991; Van der Zee et al., 1992, 1995b; Sunayashiki-Kusuzaki et al., 1993). Moreover, mouse hippocampal PKC activity correlates positively with the ability to learn a spatial discrimination task (Wehner et al., 1990). Besides our findings that confirm results of previous reports (Beldhuis et al., 1992; Van der Zee et al., 1992), the present results also show strong enhancement of PKC-ir, particularly in the posterior hippocampal CA1 and CA3 regions. We do not know whether altered PKC-ir following training reflects changes in antigenicity or changes in molecular configuration. Observations by Van der Zee et al. (1992) suggest that the enhanced PKC-ir following hole board training may result from conformational changes of PKC, possibly as the result of activation of the protein. This notion is in agreement with the observation that the total amount of hippocampal PKC-ir is not altered after associative learning (Van der Zee et al., 1995b). In a recent survey study, evidence was reported to support the idea that PKC-ir represents binding of the 36G9 antibody to exposed binding sites evoked by molecular unfolding after cellular activation (Van der Zee et al., in press).

Interestingly, we observed that stress itself significantly increased PKC-ir in the CA3 area and the DG molecular and granular cell layers of the anterior hippocampus. Activation of PKC in the hippocampus after stress may be explained by the stress-related release of glutamate (Moghaddam, 1993). Stimulation of metabotropic glutamate receptors activate PKC through hydrolysis of phosphatidyl inositol. In addition, activation of NMDA receptors by glutamate and the subsequent increase of

![Figure 8](image1.png)

**FIGURE 8.** Optical density (OD) of PKC-ir (A) in the CA1 and CA3 pyramidal cell layer and (B) in the dentate gyrus molecular layer (ML) and the inner (DG in) and outer (DG out) blades of the dentate gyrus granular cell layer in the anterior hippocampus (I.A. 5.7 according to Paxinos and Watson, 1982). No significant differences in the distribution of PKC were observed between either group. Naive (n = 6), trained (n = 7), corticosterone-treated (n = 5), and corticosterone-treated/trained (n = 6) are shown. The insert at the top indicates the selected regions for measuring the OD.

![Figure 9](image2.png)

**FIGURE 9.** Optical density (OD) of PKC-ir (A) in the CA1 and CA3 pyramidal cell layer and (B) in the dentate gyrus molecular layer (ML) and the inner (DG in) and outer (DG out) blades of the dentate gyrus granular cell layer in the posterior hippocampus (I.A. 4.2 according to Paxinos and Watson, 1982). Training enhanced PKC-ir in control and corticosterone-treated rats. Asterisks indicate statistically significant differences as compared with the naive group (P < 0.05). Naive (n = 6), trained (n = 7), corticosterone-treated (n = 5), and corticosterone-treated/trained (n = 6) animals are shown. The insert at the top indicates the selected regions for measuring the OD.
intracellular Ca\(^{2+}\) may potentiate the Ca\(^{2+}\)-dependent activation of PKC\(\gamma\). The enhanced PKC\(\gamma\)-ir following stress was not observed in stressed animals that were subsequently trained. However, in the stressed animals, PKC\(\gamma\)-ir was determined directly after the 8 days of stress; in the stressed animals that were subsequently trained, the PKC\(\gamma\)-ir was measured 16 days after termination of the stress experience. Therefore, the blunted PKC\(\gamma\) response of ST animals may be difficult to interpret because of the difference in survival time after the stress. It is tempting to speculate on whether the regional differences in PKC\(\gamma\)-ir after stress (anterior hippocampus) or training (posterior hippocampus) indicate differential contribution of these regions to sensory processing and/or memory (Amaral, 1993).

Animals that were stressed or treated with corticosterone prior to training showed a similar distribution pattern of PKC\(\gamma\)-ir in the posterior CA1 area when compared with trained animals. This finding suggests that 8 days of subordination stress, which causes posterior CA1 area when compared with trained animals. This development of reference and working memory. Moreover, the distribution of PKC\(\gamma\)-ir in ST animals showed a similar distribution pattern of PKC\(\gamma\)-ir in the anterior hippocampus, which may be related to a decreased PKC response of posterior CA3 pyramidal neurons (Woolley et al., 1990; Watanabe et al., 1992; Luine et al., 1994; Magarinos and McEwen, 1995, McEwen and Sapolsky, 1995). Nevertheless, it remains to be investigated whether the 8 days of subordination stress causes dendritic atrophy. Besides, it is challenging to establish the relationship between altered memory performance, a decreased PKC response of posterior CA3 pyramidal neurons, and the cytology of CA3 apical dendrites. In this respect, the link between PKC\(\gamma\) and its effect on cytoskeletal protein dissociation might be an important observation (Tsuyama et al., 1986).

In conclusion, the present results demonstrate that prolonged psychosocial stress causes spatial learning deficits, whereas the elevation of corticosterone to stress values cannot solely account for spatial memory impairment. The spatial learning deficits following stress are reflected only in part to the redistribution of hippocampal PKC\(\gamma\) following training, suggesting that PKC\(\gamma\) is only partly responsible for plasticity changes during and following memory formation. An interaction between corticosterone and other stress-related factors as demonstrated by a number of studies is likely to be involved in the presently observed stress-induced cognitive deficits (Bohus, 1994).

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