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REPEATED BLOCKADE OF MINERALOCORTICOID RECEPTORS, BUT NOT OF GLUCOCORTICOID RECEPTORS IMPAIRS FOOD REWARDED SPATIAL LEARNING

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SUMMARY

Corticosteroids from the adrenal cortex influence a variety of behaviours including cognition, learning and memory. These hormones act via two intracellular receptors, the mineralo-corticoid receptor (MR) and the glucocorticoid receptor (GR). These two receptor types display a high concentration and distinct distribution in the hippocampus, a brain region which is directly involved in the regulation of spatial orientation and learning. In this study, repeated subcutaneous administration of the mineralocorticoid receptor antagonist RU28318 (1.0 mg/100 g body weight), the glucocorticoid receptor blocker RU38486 (2.5 mg/100 g body weight), or a combination of both antagonists were investigated for their effects on working—and reference memory in morning and afternoon trials during 8 subsequent days in food rewarded spatial learning in a hole board task. Each rat received one dose of either vehicle (2% ethanol in PEG 400), RU28318, RU38486 or the combination of both antagonists directly after the first trial on training days 1, 3, 5, and 7. The experiments demonstrated that repeated blockade of mineralocorticoid receptors impairs reference memory reflected in the morning—as well as in the afternoon trial, whereas blockade of glucocorticoid receptors has little effect on this type of cognitive behaviour. Furthermore, combined blockade of MRs and GRs resulted in a decrease, in both daily trials, in reference memory as well as working memory performance. These findings suggest that in this spatial learning paradigm, the impairment of working memory required blockade of both receptor types, while reference memory performance involves predominantly the mineralocorticoid receptors.

Keywords—Hippocampus; Glucocorticoid receptors; Spatial learning; RU38486; RU28318; Central nervous system.

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INTRODUCTION

There is a large body of evidence that the mammalian hippocampus is crucially involved in cognitive functions such as learning, memory and spatial orientation (Eichenbaum et al., 1990; Nadel, 1991; Van der Zee et al., 1992). The hippocampal formation is necessary to learn the location of objects or to learn to navigate in a spatial orientation task (Jarrard, 1995; Morris, 1981; Schenk and Morris, 1985).The high density of receptors for corticosterone (CORT) in the hippocampus suggests that corticosteroids are important modulators of hippocampal functions and that these receptors are liable to mediate corticosteroid effects on cognition, learning and memory.

The adrenal hormone corticosterone binds to two distinct, intracellular receptors, that mediate CORT induced changes in gene expression. The mineralocorticoid receptor (MR), which displays a high affinity for CORT ($K_{d,cort} \sim 0.2-0.3$ nM) and the glucocorticoid receptor (GR) with a relatively low CORT affinity ($K_{d,cort} \sim 3-5$ nM). Low (basal) circulating levels of CORT therefore predominantly bind to MRs, whereas GRs become substantially occupied during elevated CORT levels as is the case during stress. Neurons in the hippocampus, septum and amygdala are richly endowed with MRs, while GRs are widely distributed and also occur with high densities in many other brain regions as well (Fuxe et al., 1985; Reul and De Kloet, 1985; Van Eekelen et al., 1987, 1988).

Mechanistic evidence for the importance of glucocorticoids in learning and memory has emerged from studies using both in vitro and in vivo models. First, acute stress produces a deficit in hippocampal long-term potentiation (LTP) (Foy et al., 1987; Shors et al., 1992), a phenomenon of synaptic plasticity that has been correlated with processes of learning and memory (Madison et al., 1991; Martinez and Derrick, 1996). Second, removal of endogenous glucocorticoids by adrenalectomy (ADX) impairs spatial orientation learning (Oitzl and De Kloet, 1992), and has a deranging effect on conditioning processes such as extinction of inhibitory avoidance learning, which can be restored by corticosterone replacement (Bohus and De Kloet, 1981). Third, elevation of circulating corticosterone concentrations by systemic administration of corticosterone or GR agonists also exerts an inhibitory influence on learning and memory (Arbel et al., 1994; Bodnoff et al., 1995; Bohus, 1994; De Kloet et al., 1988; Kerr et al., 1991). Fourth, long-term glucocorticoid exposure resulted in an impaired maze learning performance (Endo et al. 1996). However, Luine et al., (1993) reported no changes in performance on the eight arm radial maze after chronic ingestion of corticosterone via drinking water. Furthermore, Sandi et al., (1997) showed in the water maze that the effect of exogenously administered corticosterone appears to be experience-dependent, with the experience-induced corticosterone concentration as a critical factor determining the cognitive consequences of steroid treatment.

Short-term and acute effects of MR and GR blockade on learning and memory have been reported by Oitzl and De Kloet (1992), Oitzl et al. (1993), however, less attention was given to the long-term effects of chronic treatment with antagonists of MRs and GRs on spatial learning. For that reason, the present study was designed to investigate 0the effects of repeated administration of MR and GR antagonist on the cognitive performance in the food rewarded holeboard learning task (Oades and Isaacson, 1987). In this spatial orientation test rats show a gradual improvement in their level of spatial orientation and learning performance expressed in reference and working memory ratio's. The experiments demonstrated that repeated blockade of MRs impairs spatial orientation, whereas blockade of GRs has little effect on this type of cognitive behaviour.

MATERIALS AND METHODS

Animals

Adult male Wistar rats ($n = 41$) (3 months of age, ± 330 g body weight) were used in the present study. The animals were group housed (six per cage) and kept on a 12 h light–dark regime (lights on between 0700 and 1900h). All animals had free access to standard rat chow and tapwater. The animals were divided into four groups: C (vehicle treated controls; $n = 8$), MR (anti-mineralocorticoid treated; $n = 8$), GR (anti-glucocorticoid treated; $n = 8$), and MRGR (treated with both antagonists; $n = 8$). The experiments were carried out during the light period (between 0900 and 1600h). The animal experiments were approved by the Committee on Animal Bio-Ethics of the University of Groningen.

Treatment

The mineralocorticoid antagonist (RU28318; 3,3-oxo-7-propyl-17hydroxy-androstan-4-en-17yl-propionic acid-lactone)(Perroteau et al., 1984) and the glucocorticoid antagonist (RU38486; 17β-hydroxy-11β-(4-dimethyl amino-phenyl)17α-(1-propynyl)estra-4,9-diene-3-one)(Gaillard et al., 1984; Moguilewski and Philibert, 1984) were provided by Roussel-UCLAF, Romainville, France. Both steroids were first dissolved in ethanol and subsequently diluted in polyethylene glycol 400 (PEG; BDH chemicals, Poole, England) until the final ethanol concentration was 2%. The vehicle control contained the same PEG/ethanol concentration. RU28318 was injected subcutaneously in a dosage of 1.0 mg/100 g body weight while RU38486 was injected in a dosage of 2.5 mg/100 g body weight (Ratka et al., 1989; Korte et al., 1996). Injections were given in a constant volume of 0.2 ml. Each rat received either vehicle, RU28318, RU38486 or a combination of both antagonists directly after the first trial on training days 1, 3, 5, and 7. This injection schedule was followed in order to reduce receptor adaptation as observed in the study of Van Haarst (1995) and to counteract chronically increased plasma corticosterone levels (Van Haarst, 1995; Ratka et al., 1989).

Since the present study focusses on the role of corticosterone through its specific receptor types on learning processes, we also measured plasma corticosterone levels immediately after the training session. For this purpose nine animals were provided with a permanent silicon catheter (0.95 mm OD, 0.50 mm ID) in the right atrium inserted via the right jugular vein as described previously (Steffens, 1969). This method allows frequent blood sampling in undisturbed, freely moving rats. The animals were allowed to recover one week from surgery. Thereafter these animals were exposed to the spatial orientation task as described below. Blood samples of 0.45 ml were taken between 5 and 7 min after onset of the morning trial. 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 $3500 \times g$ and stored at -20°C for the corticosterone assay. Corticosterone was extracted from 75 μl plasma and determined by HPLC with UV detection at 254 nm according to Dawson et al., (1984) with minor changes. Briefly, plasma samples were deproteïnized with methanol and centrifuged. The supernatant was further cleaned by extraction on a C8 Solid Phase Extraction Column (J.T. Baker, Deventer, The Netherlands). Corticosterone was eluted with acetone and this extract was aspirated and redissolved in 25% acetonitrile/water for subsequent injection onto the column (Nucleosil, length 10 cm, i.d. 3 mm, particle size 5 μm; Chrompack, Middelburg, The Netherlands).

The mobile phase was made by mixing 340 ml Acetonitrile to a total volume of 1 l with water and was pumped at a rate of 0.5 ml/min. Dexamethasone was used as the internal standard. The absolute detection threshold for corticosterone in plasma was 8 ng/ml. The intra- and interassay coefficients were 3 and 8%, respectively.

Spatial Orientation Task

All subjects were trained in a spatial discrimination task using a hole board described originally by Oades and Isaacson (1987). The hole board is composed of a square arena ($70 \times 70 \times 45$ cm) made of PVC, containing four rows of four equidistant holes (14 cm apart, 3.5 cm diameter, depth 3 cm) in the floor plate (Oades, 1981). A start-box was attached to one of the PVC walls of the hole board. Each hole was supplied with chocolate chips covered by a replaceable, perforated false bottom to mask potential odour cues emanating from the reward in the baited holes. Thus, the rats were unable to discriminate between baited and unbaited holes by orientating on olfactory cues in the hole board in the training phase of the test. The hole board was placed in a dimly illuminated room that contained a number of distinctive extra visual cues to enhance spatial orientation. Prior to training the rats were familiarized with the chocolate chips in their home cages. Since every animal readily ate the chips, the rats were not food deprived to the holeboard procedure. Subsequently, the rats were habituated to the hole board in two 3-min trials on 5 consecutive days. During this habituation period all holes were baited with an accessible chocolate chip. A trial was started by placing the rat in the start-box. After 10 s, a guillotine door was lifted, giving the rat free access to the hole board. Once the rat had entered the arena, the guillotine door was lowered. When animals had not left the startbox within 2 min they were gently pushed into the arena. Between two trials the floor of the startbox and hole board arena was cleaned with a wet and a dry cloth. After the 5-day habituation period the animals were subsequently exposed to two daily training trials, one in the morning and one in the afternoon (inter-trial interval 3 h), on 8 successive days. In the training trials a fixed set of four holes arranged in a symmetrical pattern were baited (Oades, 1981). A hole visit was scored when the rat entered its nose in a hole. Hole visits were registered manually using a computer assisted recording device. A trial was terminated either when 3 min had elapsed and not all sweets eaten, or when the rat had found and consumed all food pellets in the baited holes. From these data the reference memory and working memory scores were calculated. The reference memory ratio was defined as the number of visits and revisits to the baited holes divided by the total number of visits and revisits to baited and non-baited holes. Working memory was expressed as the ratio of the number of food rewarded visits to the number of visits and revisits to the baited holes.

Statistics

Changes in memory performance were assessed by a two way ANOVA with repeated measures using the repeated application of GR or MR antagonists or a combination of both antagonists as a between-subject factor, and the consecutive training sessions as a within-subject factor. This overall analysis was completed by a posthoc Dunnett's test. A probability level of $p < .05$ was taken as statistical significance for all tests. All data are presented as means with their standard errors (SEM).

RESULTS

Plasma Corticosterone

Basal levels of plasma corticosterone (3.8 ± 1.4 μg/dl) were measured under resting conditions in non-habituated rats (Fig. 1). During the first introduction day in the hole board the corticosterone concentration increased to a peak level (13.1 ± 1.5 μg/dl). During the following days the corticosterone concentration gradually declined until baseline level which was reached on day 5 of the training period.

Spatial Orientation in the Hole Board

The effects of repeated application of GR or MR antagonists or a combination of both antagonists on spatial learning performance were measured on reference memory (efficacy of recall between successive trials) and working memory (immediate recall during each trial). These two parameters responded differently to receptor blockade as described below.

The performance of the cannulated animals in which plasma corticosterone was measured (Fig. 2) was similar to that of the vehicle-injected control group.

Development of reference memory ratio (RM) and working memory ratio (WM) for C, MR, GR and MRGR treated and trained animals are presented in Fig. 3. Fig. 3(A) shows the change in RM ratios as a result of spatial discrimination training in the hole board during the morning session while Fig. 3(B) shows the results of the afternoon trials. The between-groups comparison indicated a significant effect on RM during both morning and afternoon trials ($(F(3, 28) = 9, 65$; $p < .001)$; $(F(3, 28) = 13, 06$; $p < .001)$, respectively. A significant treatment by time interaction demonstrated that the treatment differentially affect the RM (morning: $(F(7, 196) = 48, 77$; $p < .001)$; (afternoon: $F(7, 196) = 41, 14$; $p < .001$). Posthoc testing revealed a significant reduction of reference memory ratio in MR and MRGR groups during morning and afternoon trials at days 4, 5, 6, 7 and 8 of the test period ($p < .05$) when compared with vehicle treated controls. No such effect was established by GR treatment since no significant difference was found between the control

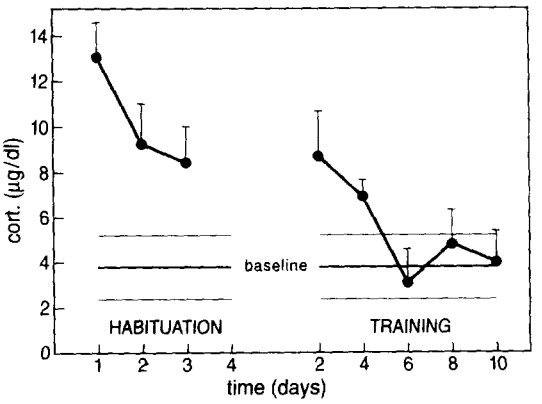

Fig. 1. Changes in plasma corticosterone during the habituation and training period of cannulated control animals ($n = 9$) in the hole board. During the 5 days of habituation only three blood samples were taken, while during the 10 days training period five samples were collected ranging from (13.1 ± 1.5 μg/dl) during the first day of habituation to (3.8 ± 1.4 μg/dl) on day 5 of the training period.

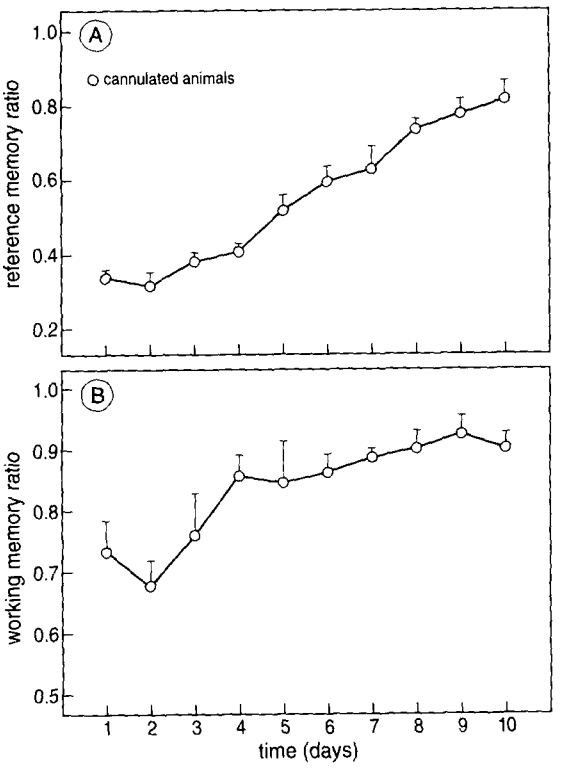

Fig. 2. Mean reference memory (A) and working memory (B) performance per day of the trained cannulated control animals ($n = 9$) on 10 consecutive days in the holeboard. The animals show a gradually inproving level of spatial orientation similar to that of the vehicle-injected control group.

and GR treated rats in the morning sessions. Only on day 5 during the afternoon sessions GR treatment produced a significant difference.

The change in WM ratios is shown in Figs. 3(C) and (D). Analysis of the measured values revealed a significant effect between controls and antagonist treated animals in the morning (C) and afternoon (D) ($F(3, 28) = 15, 07$; $p < .001$); ($F(3, 28) = 26, 95$; $p < .001$)) respectively. Furthermore, a significant group-by-day interaction was found (($F(7, 196) = 5, 94$; $p < .001$); ($F(7, 196) = 13, 92$; $p < .001$) which indicates that repeated blockade of these receptors affected WM. Posthoc testing indicated differences in WM at days 4, 6 and 7 for MR and at days 4, 5, 6, 7 and 8 for MRGR blockade ($p < .05$) as compared with control levels in the morning. Furthermore, in the afternoon a significant reduction of working memory ratio was found at day 4, 6 and 7 for MR and 4, 6, 7 and 8 for MRGR. However, there was a striking difference between the rate of WM acquisition in MR and MRGR treated rats. Although MR treatment apparently delayed the acquisition of the WM, the MR group reached control values of WM at the end of the training period. Combined MRGR blockade on the other hand significantly attenuated WM.

Total visits of baited and nonbaited holes and number of correct visits are shown in Table I. The decrease in the total number of visits in combination with an increase in the correct visits of baited holes are indicating that the choice accuracy is improved during the testing period for the C and the GR treated groups and less for the MR and MRGR treated groups.

DISCUSSION

The present study demonstrates that repeated blockade of MR impaired reference memory in a hole board learning paradigm. Combined blockade of MRs and GRs resulted in a similar decrease in reference memory performance, whereas GR blockade alone failed to affect cognitive performance in this spatial learning task. Furthermore, anti-MR treatment suppressed the working memory acquisition rate in the initial phase of the training period, whereas working memory scores of the anti-MR/anti-GR blockade were significantly lower throughout the whole training period compared to the control treated group. These results indicate the importance of MR occupation in the processing of reference memory, while optimal working memory involves activation of both MRs and GRs.

Also morphological and electrophysiological data point to the important role of MR-mediated effects of corticosterone in the hippocampus (Joëls and De Kloet, 1992; Karst et al., 1993; Sloviter et al., 1993). Low glucocorticoid levels or MR agonists enhance

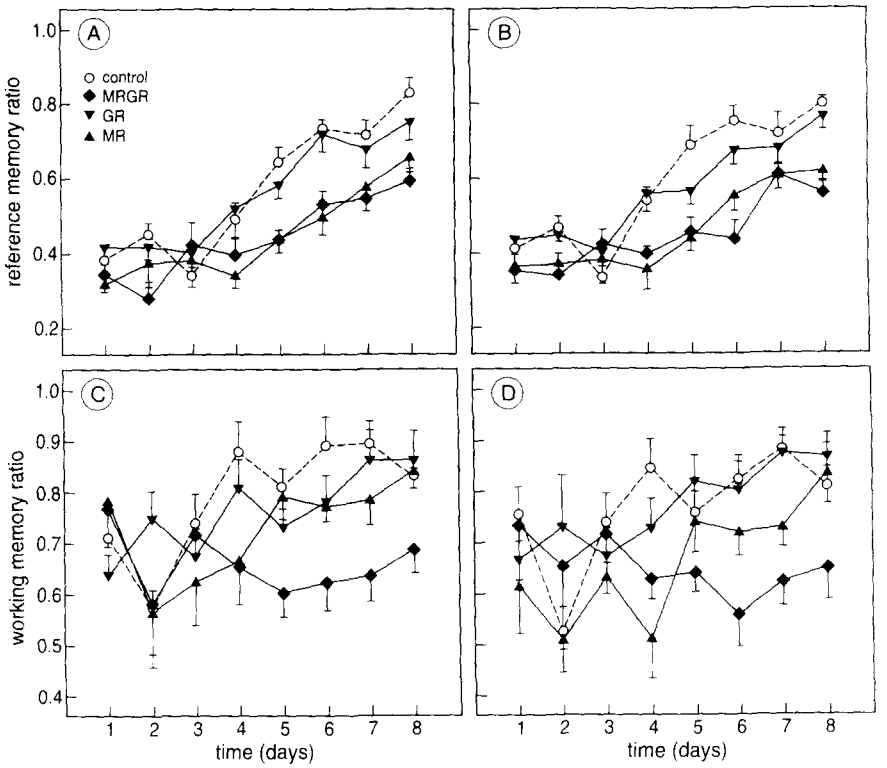

Fig. 3. Development of reference memory ratio in the morning (A) and afternoon sessions (B) of control (C, $n = 8$) and antagonist-treated animals (MR, $n = 8$; GR, $n = 8$; MRGR, $n = 8$). The between-groups measurements revealed a highly significant difference in reference memory ratio (A, B; $p < .001$). Analysis of the measured values revealed a significant difference between controls and MR and MRGR treated animals ($p < .05$). Development of working memory ratio in the morning (C) and afternoon sessions (D) of control (C, $n = 8$) and antagonist-treated animals (MR, $n = 8$; GR, $n = 8$; MRGR, $n = 8$). The between-groups measurements revealed a highly significant difference in working memory ratio (C, D; $p < .001$). Analysis of the measured values revealed a significant difference between controls and MR and MRGR treated animals ($p < .05$).

Table I. Total visits of baited and nonbaited holes compared with correct visits of baited holes during the training phase

	Day	Control		MR/GR		GR		MR	
		Total visits	Hits of baited holes	Total visits	Hits of baited holes	Total visits	Hits of baited holes	Total visits	Hits of baited holes
A	1	18.38 (1.20)	3.75 (0.16)	9.25 (0.72)	2.50 (0.42)	18.00 (0.97)	3.63 (0.18)	14.25(1.19)	3.13 (0.44)
	2	15.75 (1.01)	3.63 (0.18)	12.63 (2.00)	2.00 (0.53)	13.50 (1.30)	3.38 (0.38)	13.13 (1.62)	2.88 (0.55)
	3	13.00 (1.04)	3.25 (0.49)	11.25 (0.98)	3.38 (0.26)	16.00 (1.17)	3.75 (0.16)	11.75 (0.90)	3.13 (0.48)
	4	10.88 (0.84)	3.63 (0.26)	13.13 (0.80)	3.13 (0.40)	11.63 (0.78)	4.00 (0.00)	12.13 (1.20)	2.38 (0.53)
	5	7.63 (0.45)	3.75 (0.25)	16.38 (1.03)	3.88 (0.13)	10.38 (0.52)	3.88 (0.13)	11.00 (0.86)	3.50 (0.38)
	6	6.38 (0.36)	3.88 (0.13)	9.63 (0.80)	3.13 (0.44)	6.63 (0.51)	3.63 (0.38)	8.25 (0.58)	2.88 (0.44)
	7	7.25 (0.41)	4.00 (0.00)	12.00 (0.71)	3.88 (0.13)	7.25 (0.39)	4.00 (0.00)	8.88 (0.72)	3.25 (0.41)
	8	5.63 (0.28)	3.75 (0.16)	8.75 (0.64)	3.25 (0.25)	6.13 (0.43)	3.63 (0.38)	6.75 (0.42)	3.50 (0.27)
B	1	9.25 (0.85)	3.00 (0.42)	10.13 (1.49)	2.00 (0.46)	13.00 (0.84)	4.00 (0.00)	11.38 (1.50)	2.63 (0.53)
	2	16.63 (0.68)	3.88 (0.13)	15.63 (1.50)	3.13 (0.40)	13.88 (1.35)	3.88 (0.13)	14.50 (1.05)	3.38 (0.50)
	3	16.25 (0.76)	3.63 (0.26)	14.63 (1.57)	3.00 (0.42)	16.50 (1.18)	4.00 (0.00)	14.50 (1.17)	3.38 (0.32)
	4	8.25 (0.73)	3.75 (0.16)	15.38 (1.35)	3.38 (0.26)	10.50 (0.74)	4.00 (0.00)	14.25 (1.69)	2.88 (0.55)
	5	7.88 (0.47)	3.88 (0.13)	14.25 (1.00)	3.75 (0.16)	9.00 (0.57)	4.00 (0.00)	11.38 (0.98)	3.25 (0.41)
	6	6.25 (0.47)	3.50 (0.27)	11.25 (1.22)	2.88 (0.58)	8.43 (0.71)	3.86 (0.13)	8.63 (0.89)	3.13 (0.48)
	7	6.38 (0.37)	4.00 (0.00)	10.50 (0.60)	3.88 (0.13)	7.38 (0.43)	4.00 (0.00)	6.75 (0.47)	3.25 (0.41)
	8	6.50 (0.20)	4.00 (0.00)	7.50 (0.67)	3.38 (0.50)	6.13 (0.37)	3.88 (0.13)	7.63 (0.59)	3.63 (0.38)

A, scores during the morning sessions; B, scores during the afternoon sessions. Data presented are mean ± SEM.

LTP and reduce after-hyperpolarization (AHP), which is associated with improvement of memory related processes such as spatial learning. High glucocorticoid levels and GR agonists had an opposite effect in reducing LTP and increasing AHP (Diamond et al., 1994; Joëls and De Kloet, 1992; Kerr et al., 1994).

Behavioural effects of GR and MR antagonists have been described in tests designed to examine learning and memory formation during place navigation in the Morris water maze (Oitzl and De Kloet, 1992; Oitzl et al., 1993). In these studies it was proposed that GRs are involved in consolidation processes, whereas MRs are more related to processes of evaluation of the situation and response selection. In addition, the latter authors concluded that the activity of central MR's affects the state of the animal, while blockade of central GRs interferes with the learning process by disrupting consolidation of spatial information.

Interestingly, Sandi and Rose (1994) investigating how MR and GR blockade during the early development of chicks affects learning performance, came to similar conclusions, that is a putative role of MRs in non-specific environmental aspects of the learning task.

The results of the present study also suggest that the two corticosteroid receptor types are implied in different aspects of the process of memory formation. MR blockade-induced impairment of reference memory may be the result of deranged processing of non-specific behavioral components, such as changes in the response to the taste of chocolate, visual characteristics of the holeboard or search strategies. Such extrinsic or intrinsic factors could result in changes in the interpretation of the task and therefore in an altered learning strategy. However, based on the current data available we cannot exclude the possibility that MR or GR blockade modifies search strategies and in this way could have interfered with specific components of the learning task.

The relatively low levels of circulating plasma corticosterone especially during the second phase of the training period suggest that the effects of corticosterone on the development of spatial memory in this task are mainly mediated through the occupation of MRs. Therefore it is not surprising that the blockade of the MR was more effective in memory impairment than the administration of anti-GR ligands. In addition, Krugers et al. (1997) reported that the impact of corticosterone pellet implantation on cognitive performance in the hole board was confined to impairment of working memory in the initial period of training. On the other hand, it should be clear that the currently used repeated but discontinuous GR blockade, does not rule out a role of GRs in the modulation of spatial memory acquisition. In this respect it may be assumed that the possible involvement of GRs in food rewarded spatial learning occurs during day 1 -4 of the early phase of training, when the corticosterone levels and thus the GR occupation are relatively high.

The level of corticosterone may possibly explain the different effects of GR blockade in the water labyrinthe or other escape tasks, and hole board learning since the levels of circulating corticosterone in the Morris test are prominently elevated (Roozendaal et al., 1996) resulting in a high occupation of both MR and GR. These higher corticosterone levels may be partly responsible for the more pronounced action of the glucocorticoid receptor antagonist in water maze learning.

Besides the direct influence of MR and GR blockade on memory mechanisms, several additional corticosteroid receptor related processes should be considered for their behavioural influences. Glucocorticoids are known to be critically involved in emotional behaviour, which may well interfere with any kind of rewarded learning paradigm

(Bohus et al., 1989; Korte et al., 1992, 1995; Silveira et al., 1993). Furthermore, Korte et al. (1996) have shown that the time interval between initial MR or GR blockade and behavioural testing is crucial for the final behavioural outcome, indicating that the role of corticosterone in learning and memory is presumably dependent on the context of the stimulus and the state of the organism. Consequently, at the time of acquisition an altered endocrine state caused by preceding experiences could interact with the storage of information. Therefore it is proposed that the changed MR/GR balance due to receptor antagonist treatment or different endocrine states leads to an altered interpretation or processing of the current information. In other words the interaction between MRs and GRs determine the effect of corticosterone on the organization of behavioural responses (De Kloet, 1991).

In conclusion, the present results by means of long-term use of corticosteroid receptor antagonists demonstrate that food rewarded spatial learning in the hole board paradigm requires at least an optimal occupation of the mineralocorticoid receptors.

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