Stress, emotion and cognition : role of mineralo- and glucocorticoid receptors

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Chapter 2

Differential MR/GR activation in mice results in emotional states beneficial or impairing for cognition

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ABSTRACT

Corticosteroids regulate stress response and influence emotion, learning and memory via two receptors in the brain, the high affinity mineralocorticoid (MR) and low affinity glucocorticoid receptor (GR). We test the hypothesis that MR- and GR-mediated effects interact in emotion and cognition when a novel situation is encountered that is relevant for a learning process. By adrenalectomy and additional constant corticosterone supplement we obtained four groups of male C57BL/6J mice with differential chronic MR and GR activation. Using a holeboard task, we found that mice with continuous predominant MR and moderate GR activation were fast learners that displayed low anxiety and arousal together with high directed explorative behaviour. Progressive corticosterone concentrations with predominant action via GR induced strong emotional arousal at the expense of cognitive performance. These findings underline the importance of a balanced MR/GR system for emotional and cognitive functioning that is critical for mental health.

INTRODUCTION

Stress and emotions facilitate or impair learning and memory processes [1]. Glucocorticoids are the stress hormones secreted from the adrenals after activation of the hypothalamus-pituitary-adrenal (HPA) axis; i.e., corticosterone in rats and mice, cortisol in humans. The effect on synaptic plasticity and memory formation is mediated by two types of nuclear receptors: MR (mineralocorticoid receptor) and GR (glucocorticoid receptor) which are located in areas involved in emotion, learning and memory. While MR is present in the hippocampus and to lesser extent in the prefrontal cortex, amygdala and paraventricular nucleus [2-5], GR can be found throughout the brain with high levels in the hippocampus and paraventricular nucleus [6]. Other characteristics are the differential affinities for corticosterone: MR has a tenfold higher affinity than GR, resulting in predominant MR occupation during low basal levels and additional GR activation during increased corticosterone concentration due to stress or circadian peak activity of the hypothalamic-pituitary-adrenal (HPA) axis [7]. The precise involvement of MR and GR in emotion and cognition is still debated.

Animal studies have shown that activation or blockade of either receptor influences behaviour related to anxiety, exploration and memory. These behaviours are linked to the limbic system and are part of the behavioural repertoire tested in spatial memory tasks but also in fear conditioning [8]. With respect to unconditioned fear-related behaviour, Smythe [9] has described that MR modulates anxiety-like behaviour of rats in the light/dark box. Oitzl et al., have shown that intracerebroventricular injection of a rather selective MR antagonist in rats influenced corticosterone-induced behavioural reactivity to spatial novelty [10]. Recent findings in mutant mice with inactivated MR in the forebrain (Cre-loxP recombination [11]), support the pharmacologically detected role of MR on the modulation of behavioural strategies. Loss of the limbic MR impaired behavioural plasticity, evidenced by a differential performance during the first exposure to learning tasks, i.e. their behavioural reactivity to novelty. In contrast, learning slopes in the Water and Radial Arm maze were not affected. This increased behavioural reactivity to novel objects was observed in the face of normal anxiety-like behaviour in the open field and elevated-O-maze [12]. Indeed, it should be clarified whether MR affects anxiety or appropriate context-dependent behavioural reactivity. Others suggest that adaptive behaviour is modulated by a combined MR/GR mediated action. An example is the inhibition of corticosterone production and thus prevention of GR activation in the face of full MR activation: this led to decreased fear-induced immobility and fear-related anxiety in rats [13]. Complementary, exogenous corticosterone application or prior social defeat increased anxiogenic behaviour in rats tested

in the elevated plus maze 24 hrs later. Antagonism of the GR in the lateral septum eliminated the anxiogenic effect [14]. Interesting in this study is the 24 hrs delay, indicating involvement of memory. Indeed, GR is implicated in memory consolidation processes, demonstrated by using GR-agonists and -antagonists in rats, chickens as well as GR mutant mice [15-20]. Calvo et al., have shown that corticosterone-induced effects on anxiety after restraint stress require both MR and GR [21]. Taken together, MR appears to be responsible for the immediate facilitative effects of corticosterone on memory acquisition, while the modulation of spatial and fear memory relies on the presence of a functional GR [22]. To disentangle the combined contribution of MR and GR to most adequate performance, we will study the functions of these receptors in a task that allows simultaneous registration of emotional and memory parameters.

How emotion and cognition affect each other is still relatively unknown. Forgas and George suggested that a stimulus first needs to be identified before the appropriate emotional response will follow [23]. Others focus more on the neurobiological process of emotion and cognition, which can be functionally, anatomically and even pharmacologically separated [24]. We hypothesise that emotion and cognition are interdependent and both will be affected by differential MR and GR activation: we propose that the two corticosteroid receptors MR and GR contribute differentially but in a coordinated way to information processing.

The aim of this study was to examine how MR and GR interact in information processing presented by emotional and learning/memory elements of a task. Next to the well known use of MR and GR antagonists, MR/GR activation ratios can be endocrinologically and pharmacologically adjusted by removal of the adrenals (adrenalectomy -ADX) and additional subcutaneous corticosterone pellet implantation. In contrast to rats, mice that undergo adrenalectomy remain to produce low concentrations of corticosterone from scattered cell groups in the vicinity of the adrenals [25-27]. Therefore, ADXed mice provide an excellent model for predominant MR activation. Different degrees of continuous GR activation can be achieved via corticosterone released from implanted pellets. We used this approach and tested mice in the modified holeboard [28] measuring behaviours that define general activity, emotions, motivation and learning and memory. Subsequent Principal Component Analysis will allow to determine the correlation between emotions and cognition.

MATERIALS AND METHODS

Animals

Forty-eight 12 weeks old male C57BL/6 mice were obtained from Charles River (Maastricht, The Netherlands). After arrival, the mice were housed individually in the experimental room with sawdust bedding, water and food *ad libitum*, at 20^0C with controlled humidity under a 12 h: 12 h light/dark cycle (lights on at 08.00 a.m.) for at least one week. To familiarize with the bait used in the modified holeboard task, all mice received a few pieces of almonds daily in the week before surgery. All experiments were approved by the committee on Animal Health and Care from the Leiden University, The Netherlands and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals.

Endocrine manipulation of MR/GR activation

Mice were randomly selected for one of the following groups and operated accordingly: (i) Sham operated (Sham), (ii) adrenalectomized mice (ADX), (iii) adrenalectomized mice with an additional low corticosterone pellet (ALC) or (iv) adrenalectomized mice with an additional high corticosterone pellet (AHC).

Surgery

Mice were gas anaesthetised with a mixture of isoflurane/nitrous oxide (4% isoflurane bolus followed by 2% isoflurane). Body temperature was kept constant at 37^0C by a heating pad. Adrenals were removed (ADX) using the dorsal approach followed by subcutaneous pellet implantation on the flank of the animal. While in rats ADX removes the endogenous source of corticosterone, in mice it clamps corticosterone to low concentrations comparable to the circadian trough of adrenally-intact mice. Accessory adrenocortical cells secrete stable amounts of corticosterone [25-27;29] that maintains extensive occupation of MR. Stress or circadian rhythm does not lead to a rise in corticosterone in ADX mice. High circulating levels of ACTH indicate the lack of GR activation; i.e., no negative feedback.

Sham operation involved the same procedures as adrenalectomy except for the removal of the adrenals. Surgery was performed between 10.00 and 12.00 a.m. and lasted maximal 10 minutes per mouse. Adrenals were removed within 5 minutes. After surgery, all mice received an additional bottle containing 0.9% salt solution. Behavioural testing started 3 days after surgery. To confirm effectiveness of the adrenalectomy and pellet implantation, plasma corticosterone levels were measured 2 days after surgery, on day 0 of the experiment and one day after the last behavioural test on day 11. Mice with

abnormal corticosterone concentrations in the blood were excluded from further analysis. This resulted in seven mice per group.

Pellet preparation

Two types of pellets were made for subcutaneous implantation: (i) a 5% corticosterone (ICN Biomedicals INC) 95% cholesterol pellet for moderate MR/GR activation and (ii) a 20% corticosterone 80% cholesterol pellet for strong MR/GR activation. All pellets weighed 100 mg, with a diameter of 7 mm and thickness of 2 mm and were home-made. Corticosterone dose was chosen following a pilot experiment in which plasma corticosterone concentrations of about 100 and 150 ng/ml for the 5 % and 20 % pellet respectively, were measured two days after implantation.

Modified holeboard testing

Setup

The modified holeboard consisted of a opaque grey PVC box (50x50x50cm) with a centerboard (37x20cm) on which 10 grey cylinders (4 cm height) were staggered in two lines [30]. Always the same three cylinders were baited with a small piece of almond on top of a grid, and marked with a white ring. Seven other cylinders contained a non-obtainable almond underneath the grid and were marked with a black ring. The mice were placed in the modified holeboard for 3 trials per day with changing start positions. One trial lasted maximally 5 min, or until the mouse had found the three baits. All testing was performed between 9.00-12.00 a.m.

Behavioural observation

The behaviour of the mice was observed, recorded and analyzed with a semi automatic scoring system (The Observer Mobile 4.1, Noldus Information Technology, Wageningen, The Netherlands). All measured behavioural parameters are represented in table 1. As indication for (i) working memory, the number of repeated holevisits was calculated and (ii) reference memory, the number of visits to non-baited holes was taken. In addition, a camera was installed above the setup to measure distance moved and velocity of the mice with an automatic tracking system (Ethovision 1.95, Noldus Information Technology, Wageningen, the Netherlands).

General experimental procedure

Mice were tested in the modified holeboard over 10 days. On days 1 to 5 and 8, the three baited cylinders were marked with a white ring as visual cue while the remaining cylinders were marked with a black ring. This allowed visuo-spatial discrimination. On days 6 and 7, mice were not tested. On days 9 and 10, all

rings were removed from the cylinders, but the bait remained in the same cylinders. This allowed to estimate if the mice used a spatial strategy or visual discrimination to solve the task.
A trial lasted maximally 5 minutes and was ended when the mouse had eaten all three baits.
On days 0 and 11, blood was collected via a tail incision or after decapitation. Blood plasma was used to measure corticosterone concentrations (ICN Biomedicals, Inc). Because exposure to high concentrations of corticosterone results in shrinkage of the thymus, thymusweight was estimated as well.

Total number	Sit
	Rearing
	Stretched attend
	Grooming
	Centerboard-entries
	Holevisits
	Baited holes visited
	Non-baited holes visited
	Repeated holevisits
	Baits obtained
Latency	First centerboard-entry
	First holevisit
	Eat bait
Time	Sit
	Grooming
	On centerboard
	To finish task

Table 1. Behavioural parameters measured in the modified holeboard.

Statistical analysis
Differences in corticosterone concentrations between groups and days were analysed by two-way ANOVA (SPSS 11.5.0) with Tukeys post hoc analysis. To analyse thymus and bodyweight differences, a one-way ANOVA was performed.
The behavioural data are presented as mean of 3 trials per day $\pm$ SEM. Data were subjected to General Linear Model (GLM) -repeated measures with Tukey as post-hoc test to analyse progression over days and group differences per day. Furthermore, factor analysis (Principal Component Analysis PCA) was performed over groups and days to obtain a more comprehensive analysis of emotional and cognitive parameters. This analysis uses cross-mouse comparisons to distinguish the relation between behavioural parameters. It includes as much data as possible in each factor to minimize residual variance from the original dataset. The PCA was performed with a Varimax rotation on

variables with communalities over 0.7, that is, of which 70 % of the variance is explained by the Factors extracted. The number of extracted Factors was not pre-defined; Factors with an Eigenvalue > 1 were accepted. Factor scores were subjected to a two-way ANOVA to determine differences between groups and days. $P < 0.05$ was accepted as level of significance.

RESULTS

Behaviour

Emotion and Exploration

Figure 1 shows the results for some of the emotional and explorative parameters during all days of testing in the modified holeboard. Figure 1A illustrates that ADX followed by ALC mice have a high percentage of time spent on the centerboard, indicative for low anxiety [31-34] during the first few days. In contrast, AHC and sham mice spent little time on the centerboard during this period. From day 4 on, few significant differences were found between groups. GLM from day 1 to 10 revealed a significant group/day interaction $F(21, 588)$ 2.355, $p=0.001$.

Figure 1B shows that AHC mice display twofold more defecation compared to other groups, indicating high arousal. With repeated testing, ALC mice display less defecation compared to ADX and AHC mice. GLM revealed a significant progressive decrease over days $F(21,588)$ 7.629 $p< 0.0001$, just passing statistical significance between groups ($F(21,588)$ 1.524 $p=0.063$).

The number of rearings was taken as measure for general exploration (figure 1C). Comparing the first and the last day of testing, no differences were found between groups while on days 2, 3 and 4 ADX mice display the lowest number of rearings. GLM showed a significant change over days ($F(21,588)$ 11,439 $p< 0.0001$) although not significant between groups ($F(21,588)$ 1.25 $p=0.203$).

ADX mice display highly directed exploration/behavioural reactivity on all days of testing, reaching statistical significance on days 1 and 2 as indicated by the number of holevisits (figure 1D). Sham, AHC and ALC mice start off with few holevisits which increase over time. GLM supported this by significant group/day interaction $F(21,588)$ 1.983, $p=0.006$.

Total distance moved and velocity was comparable between groups over all days of testing (data not shown).

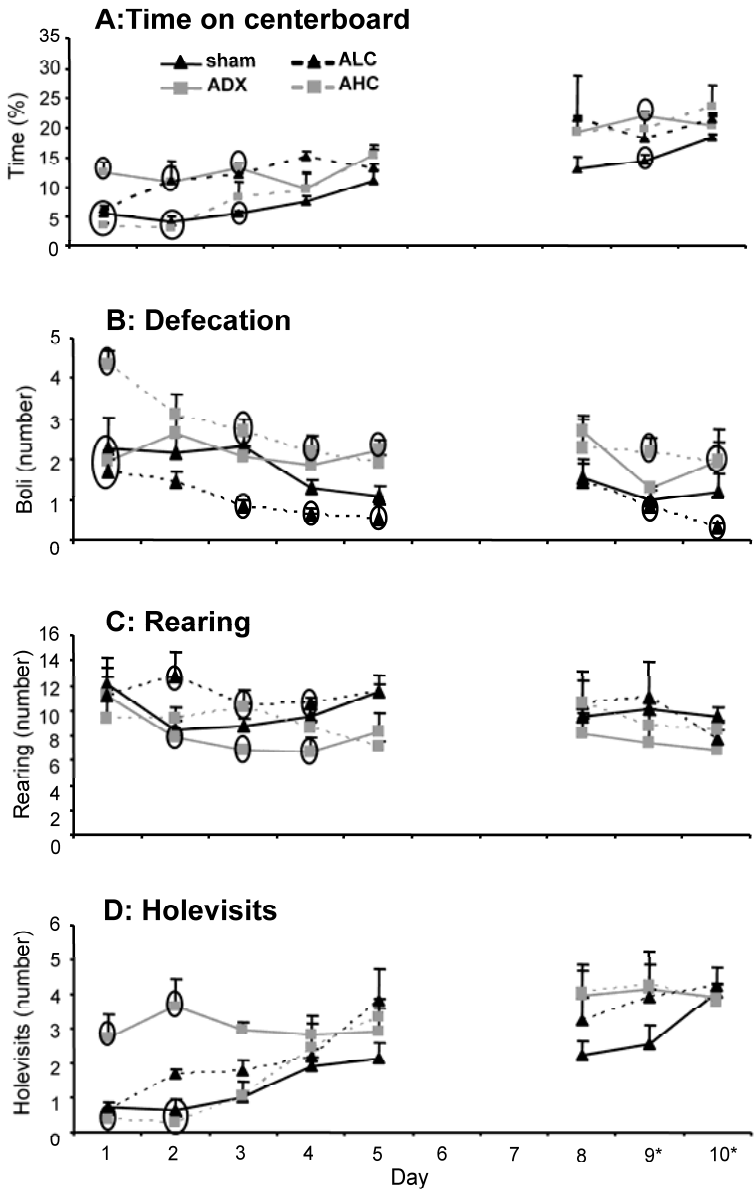

Figure 1. Behaviour of mice in the modified holeboard. (A) Percentage of time spent on centerboard (B) Number of defecation. (C) Number of rearings. (D) Number of holevisits, including revisits of Sham (black line), ADX (grey line), ALC (striped black line) and AHC mice (striped grey line). * at days 9 and 10 on the X-axis indicate removal of rings from all cylinders, while the bait remained in the same cylinders as before. Data present the mean of the three trials per day $\pm$ SEM. Ovals mark data points with significant differences $p < 0.05$ between groups within days.

Cognition

Figure 2 shows the results for three cognitive parameters on all days of testing in the modified holeboard. Figure 2A illustrates increased repeated holevisits (working memory) in ADX mice on day 8 of testing compared to Sham mice. We consider the low repeated holevisits on days 1 and 2 of Sham, ALC and AHC mice as not reliable, because the total number of holevisits is also very low on these days. Over time, Sham, ALC and AHC mice show increased repeats in parallel with increased total holevisits. GLM showed a significant group/day interaction ($F(21, 532)$ 2.029, $p=0.005$).

Figure 2B shows no significant differences in non-baited holevisits (reference memory) between Sham, ADX, ALC and AHC mice during all days of testing.

The time to finish the task is an additional learning parameter (Fig 2C). ADX and ALC mice were fast learners compared to Sham and AHC mice. Removal of the rings on days 9 and 10 did not influence the time to finish the task, indicating the use of a spatial learning strategy at that time of training. At the last day of testing, performance of Sham mice was still poor although progression over days proved to be significant ($F(21,532)$ 18.327, $p=0.000$).

Factor Analysis

Principal component analysis (PCA) over all behavioural data resulted in the extraction of four factors (Table 2) which explain 81% of total variance.

Factor 1 (41%) combines behavioural parameters that can be classified as anxiety, motivation and good learning, Factor 2 (19%) represents directed exploration, behavioural reactivity and working memory, Factor 3 (11%) represents general activity and Factor 4 (10%) includes behavioural parameters that can be classified as impaired learning.

One-way ANOVA between groups on factor loadings for Factor 1 (anxiety, motivation, good learning) revealed significant differences between Sham mice compared to ADX, ALC and AHC mice ($F(3,279)$ 11.562, $p=0.000$). Significant group differences were also found between ADX mice compared to Sham, ALC and AHC mice for Factor 3 (general activity; $F(3,279)$ 8.362, $p=0.000$).

Furthermore, when comparing the factor loadings over days, significant differences were found for Factor 1 between days 3 and 4 compared to days 9 and 10, ($F(7,279)$ 4.460, $p= 0.000$). This indicates low anxiety, more motivation and better learning at the end of testing in all groups. Factor 3 was significantly different between day 2 and days 1, 8 and 9 ($F(7,279)$ 2.522, $p= 0.016$), which indicates that general activity was decreased at the end of testing.

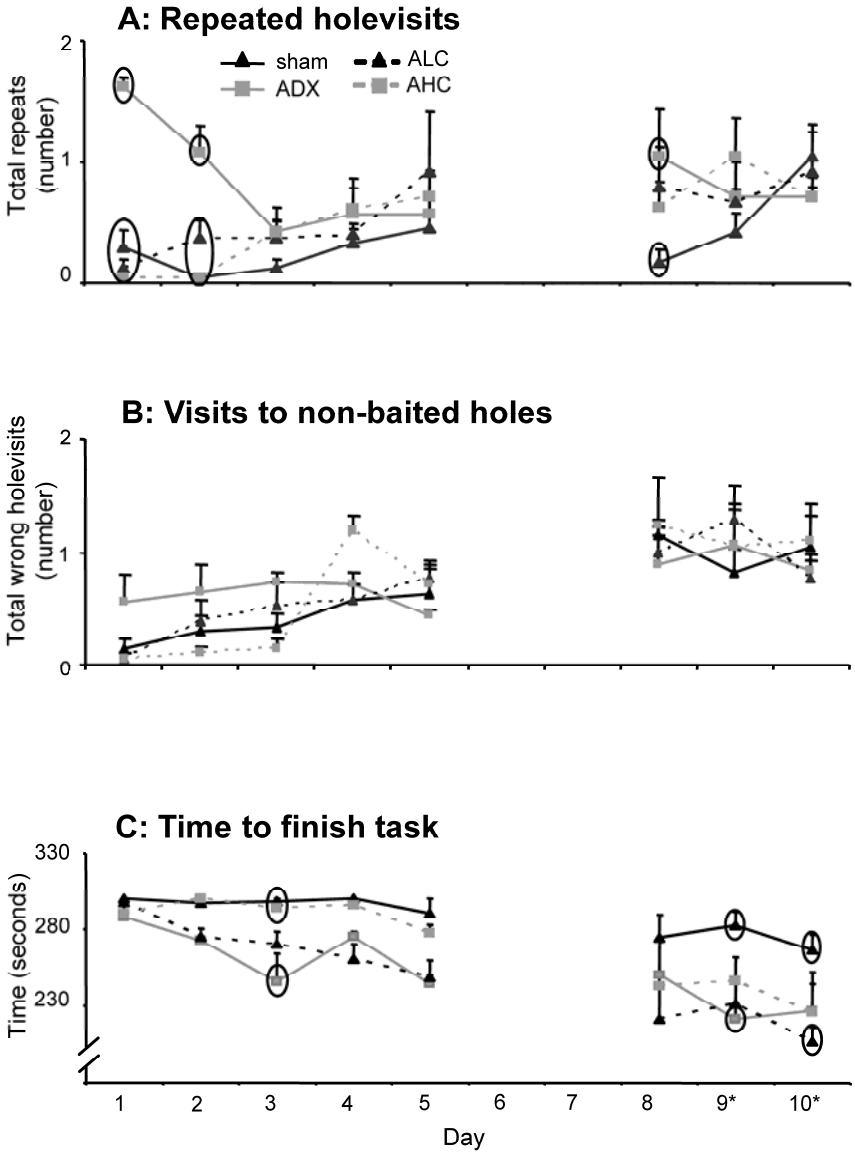

Figure 2. (A) Working memory expressed as number of holes revisited. (B) Reference memory expressed as visits to non-baited holes. (C) Time to finish the task, i.e., to obtain all three baits or 5 min, of Sham (black line), ADX (grey line), ALC (striped black line) and AHC mice (striped grey line). * at days 9 and 10 on the X-axis indicate removal of rings from all cylinders, while the bait remained in the same cylinders as before. Data present the mean of the three trials per day $\pm$ SEM. Ovals mark data points with significant differences $p < 0.05$ between groups within days.

	FACTOR			
	1 anxiety, motivation, good learning	**2 directed exploration/behavioural reactivity, working memory**	**3 general activity**	**4 impaired learning**
Latency to eat bait	-.887			
No. of baits obtained	.862			
Latency to first holevisit	-.792			
No. baited holes visited	.781			
Time on centerboard	.678			
No. repeated holevisits		.927		
No. holevisits		.807		
Time sitting			.840	
No. rearing			-.810	
No. non-baited holes visited				.911
Ratio of % right holevisits/ % wrong holevisits				-.723

Table 2. Principal component analysis over all data, with Varimax rotation and Kaiser normalisation. Behavioural parameters are represented as factor loading per factor. Factor loadings with equal value are positively correlated, while loadings with opposing values are negatively correlated. Loadings < 0.6 are not included in this table. Eleven of the seventeen measured parameters (Table 1) have communalities > 0.7 and are included in the factor analysis. Abbreviation, No. indicates number of.

Corticosterone and Thymus weight

Plasma corticosterone and thymus weights are presented in Table 3. Both low and high corticosterone pellet groups, ALC and AHC, had higher plasma corticosterone concentrations on day 0 ($F(3,31)$ 29.540, $p=0.0001$) than the SHAM and ADX mice. On day 11 of the experiment, only AHC mice showed

significantly increased corticosterone levels (F(3,31) 28.977, p= 0.0001), compared to Sham, ADX and ALC mice. Plasma corticosterone in sham and ADX mice remained at the same low basal morning level throughout the experiment, while corticosterone concentrations of ALC and AHC mice decreased in the course of the study (F(1,15) 7.835, p= 0.014 and F(1,15) 13,344, p= 0.003).
Thymus weights on day 11 supported the exposure to elevated corticosterone during the experiment with significantly lower thymus weights for ALC and AHC mice compared to Sham and ADX mice (F(3,31) 22.332, p=0.000). In fact, ADX mice had an enlarged thymus. ALC mice had a less shrunken thymus than AHC mice, indicating exposure to lower corticosterone concentrations than AHC. Bodyweight on day 11 was comparable between groups F(24, 27) 1.731, p=0.187.

	Plasma Corticosterone (ng/ml)		**Thymus weight (mg)**	**Bodyweight (g)**
Group	**Day 0**	**Day 11**	**Day 11**	**Day 11**
Sham	13.78 ± 2.37	17.96 ± 4.10	49.3 ± 0.9	25.1 ± 0.8
ADX	12.39 ± 1.50	15.24 ± 8.81	64.2 ± 2.5 *	27.4 ± 0.7
ALC	88.67 ± 19.26 *	33.18 ± 4.87	38.9 ± 0.5 *	24.7 ± 0.7
AHC	168.00 ± 19.23 *	88.63 + 10.58 *	21.2 ± 1.2 *	25.3 ± 1.2

Table 3. Plasma corticosterone, thymus and bodyweight. Corticosterone was measured before the first day of testing (day 0) and 24 hrs after the last testing day (day 11). Data are presented as mean ± SEM. * p< 0.05 compared to all other groups.

DISCUSSION

Four groups of mice were generated by endocrine manipulation, resulting in different amounts of circulating corticosterone concentrations in the blood. Given the different affinity of the receptors for the hormone, we expect a differential MR/GR activation in these groups: (i) Sham mice with an intact HPA axis, (ii) ADX mice with residual stable low corticosterone levels and thus continuous MR activation, (iii) ALC mice with moderate elevated circulating corticosterone concentrations allowing extensive MR and moderate GR activation and (iv) AHC mice with a full MR and a substantial GR activation due to high circulating levels of corticosterone. We found emotional expressions and cognitive performance related to differential corticosteroid receptor activation. Continuous predominant MR activation directed emotional components indicative for less anxiety to the benefit of cognition, while continuous additional GR activation was associated with impaired learning.

Continuous predominant MR activation results in emotions that can be beneficial for learning

Mice with stable predominant MR activation (ADX) show increased directed exploration/ behavioural reactivity towards the cylinders (holevisits) and low anxiety during the first days of testing, i.e., when the setting is novel. This corresponds to the observation that transgenic mice with low GR, and rats with ICV injection of GR antagonist express low anxiety related behaviour [35;36]. However, it contrasts previous findings that GR blockade by single infusion of RU38486 into the hippocampus has no anxiolytic effect in rats in the light/dark box [37]. Of course, the methods to achieve predominant MR activation differ in the history of inactivated GR, species, stressed state of the animals and behavioural task. Also a differentiation between context related behavioural reactivity and anxiety is not possible. However, the design of the present study allows to make this distinction. Factor analysis reveals that the variables time on centerboard (anxiety, motivation, good learning; Factor 1) and holevisits (directed exploration and behavioural reactivity; Factor 2) and are not correlated. Thus, the general idea that mice which are more prone to go to the unprotected center area are likely to display more cylinder directed behaviour is not supported. In contrast, anxiety is correlated with motivation (latency to first holevisit, latency eat bait): mice with a low anxiety approach the unprotected area faster.

Overall, low anxiety and high directed exploration/behavioural reactivity could be beneficial for the onset of learning, especially during the first days of testing. We observed an apparent fast onset of learning in these mice with predominant MR activation. High directed exploration towards the cylinders will eventually result in finding all baits, without any necessary learning of the task. Indeed, mice of this group show an increase in working memory errors (revisits) after the two days break without testing. GR is expected to promote the consolidation of MR-related adaptive behaviour, leaving the lack of GR activation as the most likely explanation for the memory deficit. The results of the Berger study [38] can be interpreted the other way round: the lack of forebrain MR resulted in working memory deficits in the water maze task because a functional GR facilitated the consolidation of non-adaptive behaviour. We conclude that the observed behaviour of animals with differential MR and GR conditions will only be understood in relation to the contribution of both receptors.

For optimal cognitive performance, not only MR but also moderate GR activation is necessary

ALC mice with MR and moderate GR activation display low anxiety during the first days of testing, general low arousal and fast learning. Corticosterone levels

in the ALC mice were continuously elevated in the range of the circadian rise, thus, would not be expected to cause damage to neurons, downregulation of MR and GR or alterations in neurotransmitters implied in cognitive impairments [39]. In fact, ALC mice with MR and moderate GR activation, showed the best cognitive performance.

Part of this improved learning and memory ability could be explained by the emotional state of the mice. Like ADX mice, ALC mice have low anxiety (and arousal) during the first days of learning which is correlated with increased motivation and good learning. Supporting our argument is the most recent finding of Herrero, that rats with low anxiety showed faster spatial learning together with increased hippocampal MR; opposite results were found in high-anxiety rats [40]. Stronger MR availability and activation might underlie the fast onset of learning, while GR are responsible for the consolidation of this context-related information [41-44]. Therefore, it is not surprising that ALC mice with a moderately activated GR display improved or normal cognitive performance compared to ADX mice with little or no GR activation throughout testing. For optimal coordination of cognition and emotion both MR and a moderate activation of GR are necessary [45;46].

Substantial continuous GR activation in addition to MR activation is associated with high emotional arousal and impaired learning

As described by many others, chronic strong GR activation caused by e.g., severe stressors or pharmacological modulation of the HPA axis, results in impaired learning and memory [47-49], reduced synaptic plasticity in the hippocampus [50], increased anxiety [51] and even depression-like symptomatology [44]. In patients suffering from depression or Cushing's disease, elevated levels of cortisol have been associated with poorer cognitive performance in verbal memory, working memory and post encoding tasks [52-54]. Furthermore, an association between cortisol level and increased fear perception has been found in patients suffering from recurring depression [55], which also indicates a modulatory role of glucocorticoids in emotional processes.

We find similar results for emotions and cognition: AHC mice with MR and continuous high GR activation have a slow onset of learning together with increased arousal and anxiety-like behaviours and suppression of directed exploration. It is not surprising that these mice display a slower onset of learning (opposite to low anxiety and fast learning as described above). At first glance, it seems surprising that when learning starts to occur, the magnitude of learning (Figure 2 C: time to finish task, slope of the learning curve) is the same in ALC and AHC mice. The change in corticosterone availability, due to the encapsulation of the pellet, is most likely responsible for the altered behaviour.

Corticosterone levels decreased over the days to concentrations in the "normal" range, i.e. comparable to circadian peak secretion and the amount of corticosterone measured in ALC mice at the beginning of testing. Thus, in AHC mice we deal with memory impairments and high emotional arousal only during specific stages of learning, namely during the first days of testing, that coincide with really high exposure to corticosterone.

The highly anxious Sham-operated control group

We used sham-operated mice that have an intact HPA axis as control group. Unexpectedly, these mice were characterized as highly anxious and little motivation, with high arousal and a slow onset and little progress of learning. Factor 1 was significantly different over time between Sham and all other groups tested: low motivation and high anxiety throughout testing days. We got the impression that the behavioural setting remained anxiogenic to these mice. Lack of exploration of the centreboard might also prevent to learn basic rules, e.g., that cylinders are baited with almonds. This and the possible role of a prolonged effect of surgery on the HPA system resulted in a follow-up experiment. We used three groups of mice (n=6 per group): (1) sham-operated mice and (2) naïve, non-operated mice received almonds in the homecage to familiarize with the bait, like the experimental groups. (3) naïve mice received almonds in the cylinders four times in the week before the modified holebard task. Sham and naïve mice without pre-exposure to the cylinders displayed similar high anxiety and slow learning as we saw before. However, after pretraining with baited cylinders anxiety decreased, motivation increased and learning improved (Figure 3).

Since surgery did not influence behaviour on the modified holeboard, incomplete recovery from the surgery is unlikely to affect performance. Using a somewhat different experimental design, comparably long times to finish the task have been reported for C57BL/6 mice (Ohl 2003; still 280 to 300 sec after eight days of training. In contrast, prior familiarization to items of the test condition reduced anxiety-like behaviour and increased motivation, which could (in part) increase cognitive performance like it was observed in ADX and ALC mice.

It is remarkable that mice without adrenals, dysregulated HPA-axis activity and additional pellet implantation "did better" compared to the relative intact Sham and naïve control groups. These findings even more underscore that (i) high anxiety and arousal has negative consequences for cognition while (ii) less anxiety, increased motivation and goal directed exploration have a positive influence on behaviour (see also [40]). We consider the role of MR in the integration of sensory information and behavioural strategies central for reduced anxiety-related behaviour.

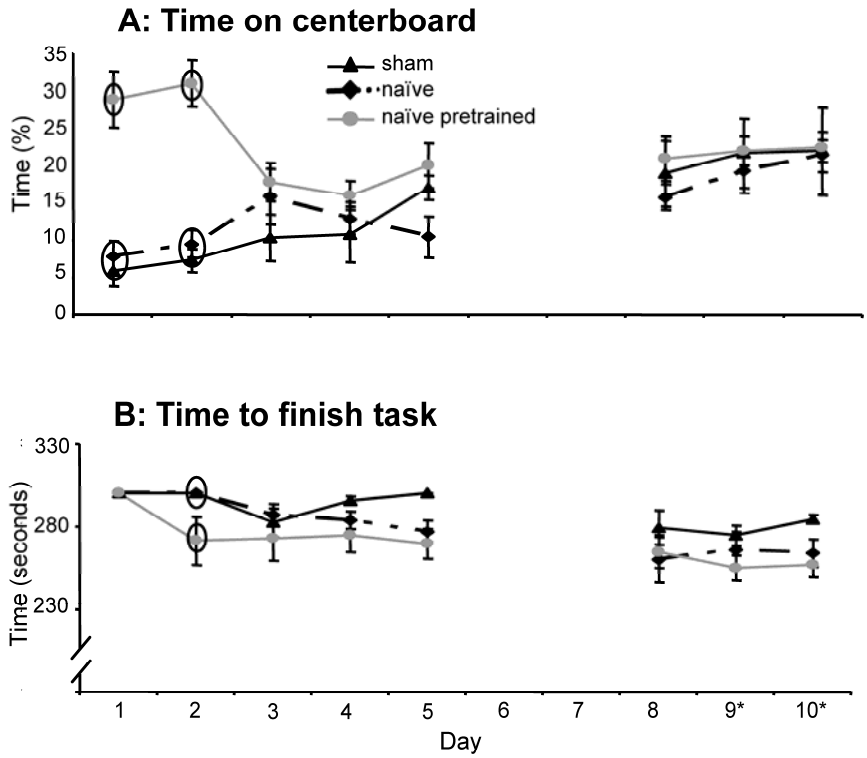

Figure 3. Examples of behaviour of the mice during the follow-up experiment. (A) Percentage of time spent on centerboard. (B) Time to finish the task (5 min or finding all three baits) of Sham (black line), naïve (striped black line) and naïve mice pre-exposed to a bait-containing cylinder in the home-cage (grey line). * at days 9 and 10 on the X-axis indicate removal of rings from all cylinders, while the bait remained in the same cylinders. Data present the mean of the three trials per day $\pm$ SEM. Ovals mark data points with significant differences: $p < 0.05$ between groups within days.

Adrenalectomy - other hormones and anxiety

The adrenalectomy-induced deficit in corticosterone secretion results in the disinhibition of HPA activity and thus enhanced release of corticotrophin-releasing hormone -CRH and vasopressin –AVP from the hypothalamus. Also the adrenal medulla as source of adrenaline is eliminated. CRH, AVP and adrenaline, all might play a role in emotional expressions and cognitive performance [56] of ADX mice, with and without supplementary corticosterone.

Considering the function of the GR in the negative feedback, we may expect that ADX mice (predominant MR activation) and ALC mice (MR and moderate GR) have a deficient suppression of CRH and AVP activity [57;58]. Mice with

elevated levels of CRH that acts predominantly via CRH receptor 1 are expected to display increased anxiety. Mutant mice with a deficient CRH receptor 1 either by genetic deletion or pharmacological blockade are less anxious [59]. Clearly, CRH is involved in anxiety-related behaviour. However in the present study, ADX and ALC mice show low anxiety-related behaviour, while AHC mice (predominant GR activation) are highly anxious. These findings do not support a role of hypothalamus-related CRH activity in anxiety behaviour in the present study. The same argument holds true for AVP.
In response to stress, noradrenalin release increases. This is thought to contribute to the anxiogenic effects of stress [60;61], in which the amygdala plays an important role [62]. AHC and Sham mice showed the strongest arousal (defecation) and were characterized as most anxious: a participation of catecholamines in these responses cannot be excluded. Furthermore, changes in metabolism and food intake have to be considered. Although food was present *ad libitum* throughout the experiment and bodyweight did not differ between the groups, motivation to go for the almond-bait might have been increased in ADX and ALC mice. Factor analysis also underlines the role of motivation in relation to anxiety for the performance.

Less directed exploration: is this anxiety?
Anxiety-related behaviour in rodents is generally deduced from the avoidance of an open, bright and unprotected area. However, tasks characteristics largely influence behaviour. For example, rats that are specifically selected for their avoidance of open arms of the elevated plus maze and thus, classified as high anxiety rats, do not avoid the center (open) area of a holeboard task [63]. Complexity and duration of the task, as well as motivational aspects might overcome state anxiety. Directed exploration or behavioural reactivity is expressed by approach to certain stimuli, e.g. the number of visits to a specific location in the testing area. These opposing behaviours are both related to locomotor activity. Does directed exploration rely on reduced anxiety? In the present study, animals with low directed exploration would spend little time near the cylinders on the centreboard. The interpretation of this behaviour could be: high anxiety. Although it is likely that anxiety interacts with directed exploration, this does not necessarily has to be the case. It could be that our interpretation of high anxiety is characteristic for a more passive exploration strategy [64;65] without a dominant role for anxiety-related behaviour. The setting of our task and subsequent factorial analysis allowed us to differentiate anxiety-like behaviour from directed exploration: they did not coincide into one factor, indicating no correlation between the two.

Conclusion

Anxiety and motivation are important factors for the onset of learning, a process in which MR and GR and their coordinated activation plays a crucial role. Continuous predominant MR activation appears to be beneficial for the emotional state, resulting in low anxiety, high motivation and high directed exploration and behavioural reactivity, but does not result in better learning and memory. Additional moderate GR activation also results in low anxiety and high motivation, with the advantage of improved cognition expressed as a decrease in working memory errors. In contrast, MR with additional substantial GR activation results in a slow onset of learning together with high anxiety, showing similarities with patients suffering from depression and Cushing's disease. We conclude that optimal performance is bound to continuous MR activation together with moderate GR activation. Further increase in corticosterone, and therefore substantial GR activation, will increase emotional arousal with impairing effects for learning and memory.

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