

Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats

P.S. Coburn-Litvak,^a K. Pothakos,^b D.A. Tata,^b D.P. McCloskey,^b
and B.J. Anderson^{a,b,*}

^a Program in Neurobiology, SUNY Stony Brook, Stony Brook, NY 11790-5230, USA

^b Department of Psychology, SUNY Stony Brook, Stony Brook, NY 11790-5230, USA

Received 22 October 2001; revised 31 December 2002; accepted 1 January 2003

Abstract

Corticosterone (CORT), the predominant glucocorticoid in rodents, elevated for 21 days damages hippocampal subregion CA3. We tested the hypothesis that CORT would impair spatial memory, a hippocampal function. In each of the three experiments, rats received daily, subcutaneous injections of either CORT (26.8 mg/kg body weight in sesame oil) or sesame oil vehicle alone (VEH). CORT given for 21 or 56 days effectively attenuated body weight gain and reduced selective organ and muscle weights. All behavioral testing was done on tasks that are minimally stressful and avoid deprivation. For each experiment, testing commenced 24 h after the last injection. CORT given for 21 days did not impair spatial working memory in the Y-maze (Experiments 1 and 2). After 56-day administration of CORT, spatial working memory was impaired in the Y-maze (Experiment 2). CORT given for 21 days also failed to impair spatial working memory in the Barnes maze (Experiment 3). However, in trials that depended solely on reference memory, the VEH group improved in performance, whereas the CORT group did not. In conclusion, CORT elevated over a period of 21 days did not impair spatial working memory, but impaired the formation of a longer-term form of memory, most likely reference memory. Impairments in spatial working memory are seen only after longer durations of CORT administration.

© 2003 Elsevier Science (USA). All rights reserved.

Keywords: Hippocampus; Glucocorticoids; Spatial learning; Y-maze; Barnes maze

1. Introduction

Stress activates the hypothalamic–pituitary–adrenal axis, ultimately causing the release of glucocorticoids from the adrenal cortex. These hormones, cortisol and corticosterone (CORT), function to maintain or restore homeostasis through energy mobilization (Chrousos & Gold, 1992), and regulation of immune responses to infection (Ruzek, Pearce, Miller, & Biron, 1999). Glucocorticoids target tissues in sense organs, internal organs, and in the peripheral and central nervous system. Within the central nervous system, the hippocampus contains a high concentration of glucocorticoid receptors and is believed to provide negative feedback, indirectly, to the hypothalamus (Dunn & Orr, 1984; Herman & Cullinan, 1997). Prolonged elevations of glucocorticoids occur in

numerous disorders, such as anorexia nervosa (Seed, Dixon, McCluskey, & Young, 2000), Cushing's disease (Starkman, Gebarski, Berent, & Scheingart, 1992), and depression (Carroll, Curtis, Davies, Mendels, & Sugarman, 1976). Glucocorticoid elevations are hypothesized to be the cause of smaller hippocampal volumes reported with depression (Sheline, Wang, Gado, Csernansky, & Vannier, 1996) and Cushing's syndrome (Starkman et al., 1992, 1999), as well as memory deficits in anorexia nervosa (Seed et al., 2000), Cushing's syndrome (Starkman, Giordani, Berent, Schork, & Scheingart, 2001), and depression (Rubinow, Post, Savard, & Gold, 1984; Sheline, Sanghavi, Mintun, & Gado, 1999). Although the complexity of these disorders precludes the investigation of cause–effect relationships between glucocorticoids, hippocampal damage, and memory deficits, such relationships can be investigated with animal models.

CORT, the predominant glucocorticoid in rodents has been shown to impair memory and damage the

* Corresponding author. Fax: 1-631-632-7876.

E-mail address: banderson@notes.cc.sunysb.edu (B.J. Anderson).

hippocampus. The shortest duration of CORT treatment tested so far, 21 days, causes no pyramidal cell loss or volume reduction in dorsal CA3 (Coburn-Litvak, Encarnacion, & Anderson, 1999), but has been reported to cause atrophy of apical dendrites in this region (Woolley, Gould, & McEwen, 1990). Any damage would be expected to result in functional deficits. For the hippocampus, those deficits would be reflected in spatial learning ability, because the ability to navigate with the use of visual spatial cues is impaired by hippocampal damage (Jarrard, 1995). We hypothesized that the same dose and duration of CORT shown to cause dendritic atrophy (Woolley et al., 1990) should also impair hippocampal function.

When testing for memory impairments associated with glucocorticoid treatment, it is important to test near the cessation of treatment because glucocorticoid-induced dendritic atrophy has been shown to be reversible (Conrad, LeDoux, Magarinos, & McEwen, 1999; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). At the same time, by testing behavior after CORT treatment rather than during treatment it is possible to avoid confounding the results with potential acute effects of the treatment. Therefore spatial memory testing in the three present experiments was performed 24 h after the end of the treatment.

Of the previous studies that have tested the effects of CORT on spatial memory, none have addressed elevations in CORT that last only 21 days. Therefore, we tested the effects of chronic CORT administration for 21 days on two tasks that require spatial learning, the Y-maze and the Barnes maze. These tasks were chosen because they avoid deprivation and overt stress, and therefore avoid confounding the results with experimentally induced differences in the need for food or water and stress response mechanisms. Rats in Experiments 1 and 2 were tested for spatial working memory deficits using the arm recognition task in the Y-maze. Conrad, Galea, Kuroda, and McEwen (1996) have shown that this 1-day spatial learning task is dependent upon an intact hippocampus. The Y-maze has three equal length arms with tall, dark walls. This task takes advantage of a rat's innate preference to explore a novel arm in a safe, dark environment. Spatial learning deficits in the Y-maze have been reported following 21 days of restraint stress (Conrad et al., 1996), which causes hippocampal dendritic atrophy (Watanabe, Gould, & McEwen, 1992). Here we tested spatial working memory after 21-day administration of CORT. Spatial learning after 56-day administration of CORT was also tested in order to determine whether longer treatment durations bring about deficits that are not apparent after only 21 days.

To be certain that the conclusions from the 21-day time point in Experiments 1 and 2 were not specific to the task used, we changed the task in Experiment 3 to

the Barnes maze. The Barnes maze takes advantage of the rats' natural preference for dark places to test memory for a position in space within the reference framework of visual spatial cues in an open environment. Performance in this maze correlates with synaptic enhancement in the hippocampal fascia dentata and perforant path (Barnes, 1979), and has been shown to be impaired after 80-day administration of CORT (McLay, Freeman, & Zadina, 1998). The same CORT-induced deficits were shown to correspond to deficits on two mazes that are known to be dependent upon the hippocampus (McLay, Freeman, Harlan, Kastin, & Zadina, 1999). In the Barnes maze memory on trials that take place shortly after the introduction of the goal position reflects working memory, a transient form of memory that is trial dependent. Performance on trials that take place 24 h later, but test for memory of the previously learned goal position reflects, in part, reference memory. Significantly, the first trial would test exclusively for reference memory (Frick, Baxter, Markowska, Olton, & Price, 1995), or a rule-based memory that is longer lasting than working memory. As a result, use of the Barnes maze with a slight modification of the procedures used by McLay et al. (1998) provides the opportunity to test both spatial working and reference memory.

2. Experiment 1

The purpose of this experiment was to test the effects of 21-day administration of CORT on spatial working memory in the Y-maze. Furthermore, we measured body, organ, and muscle weight to establish that the CORT dose used (26.8 mg/kg body weight) provided well-known peripheral effects of elevated CORT.

2.1. Methods

2.1.1. Subjects

Fifteen male Sprague-Dawley rats (250–300 g) were habituated to the colony for 2 weeks and handled for 5 days prior to the start of injections. Rats were divided so that weight was equally balanced across the CORT and VEH groups. Two of the three groups received one subcutaneous injection daily for 21 days, administered 1 h after the onset of the light cycle (12 h on:12 h off). The CORT group ($n = 5$) received 26.8 mg/kg body weight of CORT (equivalent to 10 mg/day) in 250 μ l of sesame oil, while the vehicle (VEH, $n = 5$) group received 250 μ l of sesame oil alone. We have previously measured this dose of CORT to produce plasma CORT concentrations of $42.63 \pm 7.22 \mu\text{g/dl}$ relative to VEH concentrations of $14.18 \pm 3.19 \mu\text{g/dl}$ (1–3 h post-injection). These plasma concentrations of CORT following injections are similar to concentrations measured in control

animals brought into a busy novel environment (unpublished observations). A third group of animals (Naïve, $n = 5$) was run at a separate time point and not given injections, but handled for 5 min daily for 21 days.

2.1.2. Y-maze

Testing in the Y-maze commenced on the day following the last injection. Testing took place during the light cycle, but in a relatively dark room. Behavioral activity and spatial working memory were measured.

The Y-maze dimensions and beam placements were as described in Conrad et al. (1996). The Y-maze was constructed of dark, opaque Plexiglas walls 32 cm high, 16 cm wide, and 50 cm long. In each of the three arms, two pairs of infrared photocells were located 21 and 42 cm from the arm ends. Customized software recorded time at the beginning and end of each infrared beam break. An arm entry was recorded when the rat broke the inner arm beam (relative to the center of the maze) followed by the outer arm beam. Arm exits were recorded when the rat broke the outer arm beam followed by the inner arm beam. The three arms were visually identical so that rats were required to use objects placed in the maze periphery for spatial navigation. Soiled bedding in the maze was stirred between rats to prevent navigation with odor cues.

For each rat, the three arms of the Y-maze were randomly pre-assigned as the “start” arm, the “novel” arm, and the “other” arm. Each rat was placed in the maze for two trials with a 4-h intertrial interval. The “novel” arm was blocked during trial 1. The rat was placed in the “start” arm and allowed to explore the “start” and “other” arms for 15 min. Following trial 1, the rat was returned to the home cage. In trial 2 all three arms were open. Each rat was placed in the “start” arm and allowed to explore all three arms for 5 min. Rats with an intact memory should spend a disproportionate amount of time exploring the previously blocked (“novel”) arm, because of their innate preference to explore a novel environment. The percentage of entries into each arm relative to the total arm entries was calculated for each rat during trial 2. Only the first 3 min were analyzed because novel arm recognition is best demonstrated within the early part of the trial (Conrad et al., 1996). The total number of entries was recorded as a measure of activity.

2.1.3. Statistical analyses

Total number of arm entries was analyzed with a one-way analysis of variance (ANOVA), with treatment (CORT, VEH, NAIVE) as the between subjects variable. To establish that the novel arm was preferred, the frequency of animals with the highest percent entries in the novel arm was compared to the frequency with the highest percent entries in the start and in the other arms using a χ^2 analysis. A one-way ANOVA for

the percent entries in the novel arm only was run with group as the between subjects factor. By comparing only novel arms across groups, we avoided violating statistical assumptions about the independence of data that would have occurred if all arms were used in the analysis.

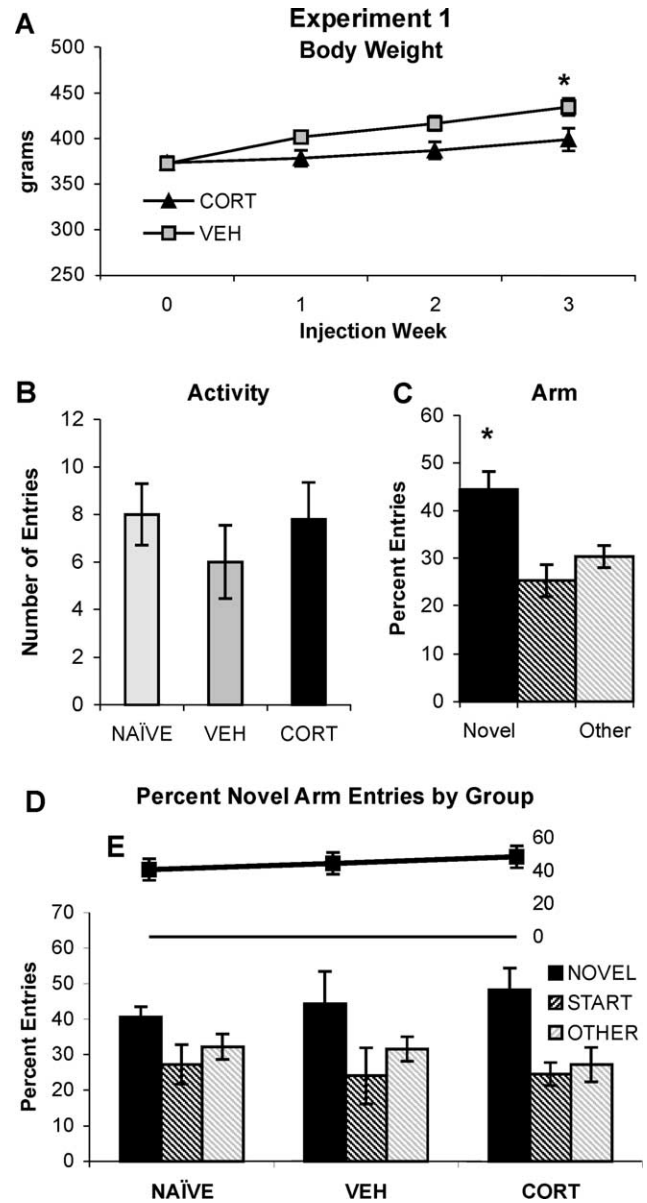


Fig. 1. Experiment 1 tested the effects of CORT given for 21 days. (A) After 21 daily injections (week 3) of VEH ($n = 5$) or CORT ($n = 5$), rats in the CORT group weighed significantly less, thereby demonstrating the ability of the dose used to produce well-known effects of glucocorticoids. (B) In the Y-maze, behavioral activity, measured as the total number of arm entries, did not differ across the CORT, VEH and Naïve groups. (C) Rats preferred the novel arm on trial 2. (D) Each group preferred the novel arm relative to entries in the start and other arms. (E) The percentage of novel arm entries did not significantly differ by group. Thus the data suggest spatial working memory is intact after 21-day administration of CORT. * $p < .05$.

2.1.4. Organ weights

Rats were killed 24 h after Y-maze testing, and the adrenal gland, heart, gastrocnemius, and soleus muscles were dissected and weighed. *t* tests were used to test for group effects on organ weights. Because the naïve animals were used in a subsequent pilot study, their organ weights were not available.

2.2. Results

2.2.1. Organ and body weights

Organ and body weight data indicated that the CORT dose given over 21 days was effective in producing classic peripheral effects of elevated glucocorticoids. Although the body weight of the two groups started out the same, the VEH group gained weight faster than the CORT group so that the CORT group weighed less than the VEH group after 21 daily injections [$t(8) = 2.27, p < .05$; see Fig. 1A]. Heart, adrenal glands, and gastrocnemius muscle all weighed significantly less after treatment with CORT compared to VEH [heart: $t(8) = 3.84, p < .01$; adrenal glands: $t(8) = 13.99, p < .001$; gastrocnemius muscle: $t(8) = 3.17, p < .05$; see Table 1]. Soleus muscle, which is known for resistance to glucocorticoids did not weigh less. When

organ weight reduction was compared to body weight reduction by analyzing organ to body weight ratios, only the adrenal gland to body weight ratio was significantly lower in the CORT group relative to the VEH group [$t(8) = 12.76, p < .001$; see Table 2], suggesting that the reduction in adrenal gland weight could not be accounted for entirely by the reduction in body weight.

2.2.2. Behavioral activity in the Y-maze

A one-way ANOVA (with group as the between subjects factor) revealed no effect of group on the total number of arms entered during the first 3 min of trial 2 [$F(2, 14) = .879, p > .05$, see Fig. 1B].

2.2.3. Spatial memory in the Y-maze

χ^2 revealed that a greater number of animals showed a preference for the novel arm than expected by chance [$\chi^2(2) = 6.2, p < .05$; see Fig. 1C]. Visual inspection of the group by test time data also supported this conclusion (see Fig. 1D). Group effects were tested by comparing the percent of novel arm entries by group with a one-way ANOVA. There was no indication that any group had impaired spatial working memory in this task [$F(2, 14) = .347, p > .05$; see Fig. 1E].

Table 1
Organ and muscle weights obtained 48 h after the last injection

	Heart (g)	Adrenal (g)	Gastrocnemius (g)	Soleus (g)
Experiment 1: 21 days				
Vehicle (<i>n</i> = 5)	1.17 ±0.04	0.0245 ±0.006	2.84 ±0.032	0.148 ±0.004
Corticosterone (<i>n</i> = 5)	1.03* ±0.01	0.0097* ±0.008	2.64# ±0.062	0.147 ±0.007
Experiment 2: 56 days				
Vehicle (<i>n</i> = 10)	1.72 ±0.106	0.0588 ±0.0097	3.199 ±0.0987	0.217 ±0.0153
Corticosterone (<i>n</i> = 10)	1.29* ±0.084	0.0226* ±0.0024	1.994* ±0.1011	0.171# ±0.0080

* $p < .01$.

$p < .05$.

Table 2
The ratio of organ or muscle weight to body weight

	Heart/body weight	Adrenal/body weight	Gastrocnemius/body weight	Soleus/body weight
Experiment 1: 21 days				
Vehicle (<i>n</i> = 5)	0.27e⁻² ±0.008e ⁻²	0.0564e⁻³ ±0.0015e ⁻³	0.656e⁻² ±0.0234e ⁻²	0.340e⁻³ ±0.0194e ⁻³
Corticosterone (<i>n</i> = 5)	0.26e⁻² ±0.012e ⁻²	0.0244e^{-3*} ±0.0024e ⁻³	0.664e⁻² ±0.0174e ⁻²	0.369e⁻³ ±0.0097e ⁻³
Experiment 2: 56 days				
Vehicle (<i>n</i> = 10)	0.29e⁻² ±0.0183e ⁻²	0.1035e⁻³ ±0.0092e ⁻³	0.544e⁻² ±0.0178e ⁻²	0.367e⁻³ ±0.02447e ⁻³
Corticosterone (<i>n</i> = 10)	0.29e⁻² ±0.0232e ⁻²	0.0493e^{-3*} ±0.0040e ⁻³	0.445e^{-2*} ±0.0223e ⁻²	0.382e⁻³ ±0.01936e ⁻³

* $p < .01$.

2.2.4. Summary of results

The effectiveness of the CORT dose (26.8 mg/kg body weight) was verified by the demonstration of a relative reduction in body weight, as well as heart, adrenal, and gastrocnemius weight. Adrenal gland weight reduction was proportionally greater than body weight reduction in the CORT group. Despite the CORT effects on organs and muscle, it caused no apparent reduction in behavioral activity or deficits in spatial working memory in the Y-maze.

3. Experiment 2

The failure to find spatial working memory deficits in the Y-maze after CORT treatment for 21 days led us to ask whether longer durations of CORT (56 days) would impair spatial working memory in the Y-maze. In Experiment 2, testing after 21-day administration of CORT was also included, because the increase in group size allowed us to ensure that the failure to detect deficits in Experiment 1 was not caused by insufficient statistical power. As a result, a CORT ($n = 10$) and VEH group ($n = 10$) were tested before treatment, and again after 21 and 56 days of treatment.

3.1. Methods

3.1.1. Subjects

Twenty male Sprague–Dawley rats were divided into two groups so that weight was distributed evenly over groups (CORT, $n = 10$; VEH, $n = 10$). Utilizing the same dose of CORT (26.8 mg/kg body weight) that was used in Experiment 1, both groups received one subcutaneous injection daily for 56 days. Vehicle was increased to 300 μ l per day for both groups. Each injection was administered 1 h after the onset of the light cycle (12 h on:12 h off).

3.1.2. Y-maze

All rats were tested at three time points: 72 h before the first injection (pre-treatment), and 24 h following the 21st and 56th injections. The 22nd injection was given after Y-maze testing so that CORT was not artificially elevated during the test. The same maze and procedures used in Experiment 1 were used for Experiment 2. The Y-maze was moved to a new room and surrounded by novel visual cues for each of the three testing time points to minimize carry-over effects.

3.1.3. Statistical analyses

Total number of arm entries, a measure of exploratory activity, was analyzed with a repeated measures analysis of variance (ANOVA), with treatment duration (pre-treatment, 21 days, 56 days) as the within subjects factor, and group (CORT, VEH) as the between subjects

factor. To determine in which condition activity differed, pairwise comparisons with a Bonferroni correction factor were used. To establish that a preference for the novel arm existed in the pre-trial, the percentages of entries into each arm was calculated. The frequency of rats that had the greatest percentage of entries for each arm was compared with a χ^2 analysis. Subsequently, a repeated-measures ANOVA was used to analyze the percent entries into the novel arm by treatment duration (a within subjects factor). By comparing only novel arms over test trials, we avoided violating statistical assumptions about the independence of data that would have occurred if all arms were used in the analysis. An effect of treatment duration was followed by pairwise comparisons using the Bonferroni correction factor to determine which, if any, durations differed.

3.1.4. Organ weights

Rats were killed 24 h after the last test session, and the adrenal gland, heart, gastrocnemius, and soleus muscles were dissected and weighed. t tests were used to test for group effects on organ weights.

3.2. Results

3.2.1. Organ and body weights

The CORT dose given over 56 days was also effective in producing classic peripheral effects of elevated glucocorticoids. Although body weight did not differ at the start of the study (see Fig. 2A), the CORT group weighed significantly less than the VEH group after 21 daily injections [$t(18) = 3.706$, $p < .01$] and after 56 daily injections [$t(18) = 6.513$, $p < .001$]. Heart, adrenal glands, gastrocnemius muscle, and even soleus muscle weighed less in the CORT group relative to VEH group following 56 daily injections [heart: $t(18) = 3.148$, $p < .01$; adrenal glands: $t(18) = 7.003$, $p < .001$; gastrocnemius muscle: $t(18) = 8.529$, $p < .001$; soleus muscle: $t(18) = 2.671$, $p < .05$; see Table 1]. When organ weight reduction was compared to body weight reduction by analyzing organ to body weight ratios, only adrenal gland to body weight, and gastrocnemius muscle to body weight were significantly lower for the CORT group relative to VEH [adrenal gland/body weight: $t(18) = 5.381$, $p < .001$; gastrocnemius muscle/body weight: $t(18) = 3.458$, $p < .01$; see Table 2].

3.2.2. Behavioral activity in the Y-maze

In Experiment 2, rats were tested before treatment, again 21 days into the treatment condition, and finally after 56 days of treatment. Activity was measured as the number of arm entries in trial 2. A repeated measures ANOVA with one within subjects factor (treatment duration: pre-treatment, 21 days, and 56 days) and one between subjects factor (group) revealed a treatment duration by group interaction [$F(2, 36) = 4.022$, $p < .05$;

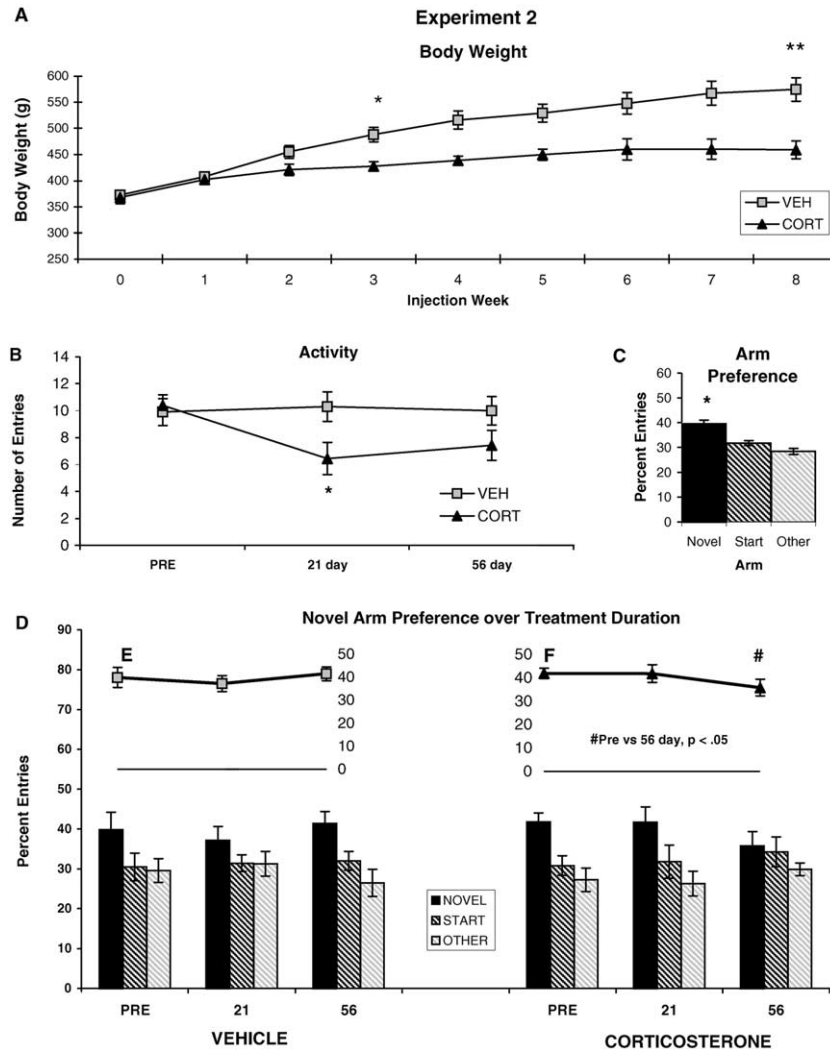


Fig. 2. In Experiment 2, rats were tested in the Y-maze prior to CORT or VEH administration, and again after 21- and 56-day administration. (A) Body weight did not differ at the start of the study, but was significantly less in the CORT group in both weeks when testing occurred, week 3 for the 21-day test, and week 8 for the 56-day test. These well-known effects of glucocorticoids confirm the effectiveness of the CORT dose administered. (B) CORT given for 21, but not 56 days reduced behavioral activity in the first 3 min of the maze when compared to activity in the PRE-treatment trial. No reduction in activity was seen at any time point of testing in the VEH group. (C) During pre-treatment, rats in both groups preferred the novel arm during the first 3 min of trial 2 in the Y-maze. This was further supported by a χ^2 analysis that compared the frequency of rats that preferred each arm. (D) Visual inspection of the data by group and time of test reveals that the percentage of novel arm entries was relatively higher at each time point except the 56-day test for the CORT condition. (E) The VEH group showed no significant difference in novel arm entries over the time of the test ($p > .05$). (F) In contrast, the CORT group had a significantly lower percentage of novel arm entries at the 56-day test relative to the PRE-treatment test, although not relative to the 21-day test. # $p < .05$, * $p < .01$, ** $p < .001$.

see Fig. 2B] with a tendency for a main effect of treatment duration [$F(2, 36) = 2.854$, $p = .071$]. A repeated measures ANOVA for the vehicle group only using treatment duration as the within subject factor revealed no effect of treatment duration. In contrast, a repeated measures ANOVA for the CORT group with treatment duration as the within subjects factor revealed a significant effect of treatment duration [$F(2, 18) = 6.087$, $p = .01$]. Pairwise comparisons revealed that the number of entries in the pre-test was significantly greater than after 21 daily injections ($p < .002$). There was a tendency for number of entries after 56 days of injections to be lower than the pre-treatment test ($p = .10$).

3.2.3. Spatial memory in the Y-maze

Because of the difference in activity levels over time, the percent entries in each arm were calculated (see Fig. 2C). Percent entries (a relative measure) leave data across arms dependent rather than independent, so we used nonparametric statistics to establish a preference for the novel arm, and then used parametric statistics to compare preference for the novel arm across test time points. First, χ^2 analysis of the frequency of rats that preferred each arm (collapsed across groups) established that there was a preference for the novel arm in the pre-treatment trial [$\chi^2(2) = 7.68$, $p < .05$]. Visual inspection of the group data over treatment duration support a

general preference for the novel arm (see Fig. 2D). Novel arm entries were then compared over treatment duration (pre-treatment, 21 days, 56 days). A one-way ANOVA for the percent entries into the novel arm for the VEH group revealed no significant difference over treatment duration [$F(1, 9) = .120, p > .05$; see Fig. 2E]. For the CORT group, the percent entries into the novel arm varied over treatment duration [$F(1, 9) = 5.166, p < .05$; see Fig. 2F]. Pairwise comparisons using the Bonferroni correction factor revealed that the percent entries into the novel arm after 56 days of treatment were significantly lower than in the pre-treatment test ($p < .05$). The percent entries in the novel arm on the 21-day test was not significantly different from either the pre-treatment or the 56-day test ($p > .05$).

3.2.4. Summary of results

CORT (26.8 mg/kg body weight) given over 56 days effectively reduced body weight gain, as well as heart, adrenal, gastrocnemius, and soleus weight. The reduction in adrenal gland and gastrocnemius weights cannot be accounted for entirely by the reduction in body weight. The conclusions drawn in Experiment 1 were supported by the finding that spatial working memory was not significantly impaired when testing took place after 21 daily injections of CORT. In contrast, CORT given for a longer time period (56 days) impaired spatial working memory in the Y-maze.

4. Experiment 3

Recognition of a novel arm in the Y-maze may be relatively easy, and therefore the ease of the Y-maze task may account for the failure to detect spatial working memory deficits after 21 daily injections of CORT. To further test spatial working memory at the 21-day time point, a third set of animals was tested in the Barnes circular platform maze. In this maze animals have to remember one goal position of 12 rather than one of three. The difficulty of this task also offered the opportunity to assess memory over several days. Consequently, reference and working memory were assessed.

4.1. Methods

4.1.1. Subjects

Twenty male Sprague–Dawley rats were divided into two groups so that weight was distributed evenly over groups. Utilizing the same dose of CORT (26.8 mg/kg body weight in 300 μ l sesame oil) used in Experiments 1 and 2, the CORT and VEH groups received one subcutaneous injection daily for 21 days, administered 1 h after the onset of the light cycle (12 h on:12 h off). Testing in the Barnes maze started on the day following

the last injection, and took place in the dark cycle over four sequential days.

4.1.2. Barnes maze testing

The Barnes maze consists of a large round platform (1.22 m in diameter) with a white surface in a brightly lit room. Visuo-spatial cues surrounded the maze periphery. In such an open environment, rats naturally seek a dark enclosed surrounding, which is provided in the form of a dark box under one of 12 evenly spaced 10-cm round holes around the perimeter of the platform. Rats were habituated to the platform on the last day of injections. During habituation, animals were first placed into the goal box. They were then placed on the platform just above the goal box and encouraged to enter the goal box. Finally, rats were placed near the center of the maze inside a rectangular alleyway leading to the goal box. Once in the goal box, rats remained there for 2 min in all phases of habituation and testing. On the day following the last injection, rats were placed into the start box, which was a hexagonally shaped enclosure that was 25 cm tall and 24 cm in diameter. Rats were placed in a random orientation relative to the goal position and remained there free to move for 30 s. The box was then raised by the investigator who stepped away from the maze to stand in the same position for each trial. Rats were then allowed to explore the maze until (a) they entered the new goal position, or (b) 3 min elapsed. Rats were returned to their home cage for a 15 min inter-trial interval while the maze and goal box were cleaned. The maze was rotated randomly to prevent rats from using odor cues to find the goal position.

Following the introduction of a new goal position (trial 0), the goal box remained in the same relative position in space for the next eight test trials. Five trials (trials 0–4) took place on day 1, and four trials (trials 5–8) took place on the following day (day 2). A second set of nine trials, using a different goal position, was performed on days 3 and 4. The time to goal box and number of errors, defined as sniffing over an incorrect hole, were recorded by an experimenter blind to the group codes.

4.1.3. Statistical analyses

Errors and time to goal box were analyzed with a repeated measures ANOVA with group (CORT, VEH) as the between subjects variable, and trials within a day as the within subjects variable. All days were analyzed separately in case reversal of CORT effects occurred over testing days. Trial 1 of days 2 and 4 reflects reference memory alone, whereas trials 2–4 on days 2 and 4 reflect both reference and working memory (Frick et al., 1995). For that reason, we compared the performance on trial 1 of days 2 and 4 to the last trial on days 1 and 3, the most conservative test of the possibility that reference memory was impaired by CORT. Both errors and

time to goal position were analyzed with a repeated measures ANOVA with trial (trial 4 of days 1 and 3 vs. trial 1 of days 2 and 4) as the within subjects factor and group as the between subjects factor. Data from 1 VEH animal were excluded from analyses because the animal failed to move on the platform. The rats were not sacrificed immediately, so the organ weights were not available for analysis.

4.2. Results

4.2.1. Body weight

In Experiment 3, the effectiveness of the CORT dose was supported by the significantly lower body weight relative to VEH [$t(17) = 4.79, p = .001$; see Fig. 3]. Despite the dramatic growth suppression, no deaths occurred in any of the three experiments.

4.2.2. Barnes maze

No significant differences were observed between groups in the number of errors on the five trials on day 1 [$F(1, 17) = .12, p > .05$; see Fig. 4A]. There was a tendency for a reduction in errors over trials [$F(4, 68) = 2.07, p < .10$], but no group by trial interaction [$F(4, 68) = .30, p > .05$]. On day 2, the CORT group made significantly more errors than the VEH group [$F(1, 17) = 5.81, p < .05$]. Errors did not change over trials [$F(3, 51) = 2.06, p > .05$], but there was a group by trial interaction [$F(3, 51) = 3.456, p < .05$]. When a different goal position was introduced on day 3, the number of errors between the two groups did not differ [$F(1, 17) = 2.23, p > .05$]. Similar to day 1, errors on day 3 decreased over trials [$F(4, 68) = 2.902, p < .05$], but there was no group by trial interaction [$F(4, 68) = .349, p > .05$]. On the following day (day 4), the CORT group relative to the VEH group made significantly more errors over four trials testing memory for the goal position that was introduced on day 3 [$F(1, 17) = 6.09, p < .05$]. Errors did not differ over trials [$F(3, 51) = .292,$

$p > .05$] and there was no group by trial interaction [$F(3, 51) = .278, p > .05$]. The pattern of findings on day 4 resembled the findings on day 2.

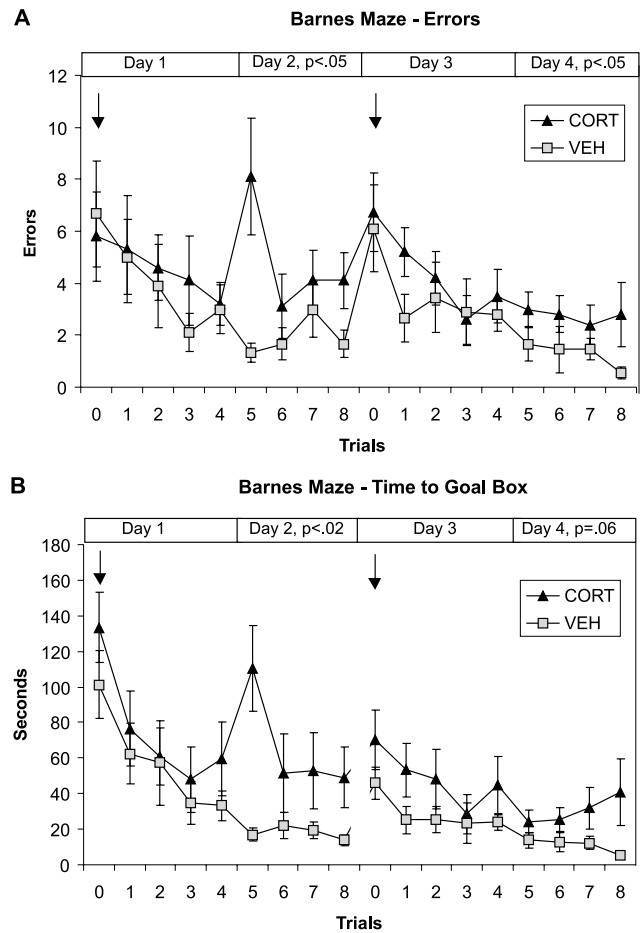


Fig. 4. Goal positions were introduced on trial 0 of days 1 and day 3 (see arrows). Four subsequent trials, within 1½ h of the introduction of the goal position, tested spatial working memory. On the following day (days 2 and 4), four additional test trials (trials 5–8) were given for each goal position. These trials included a reference memory component in trials 5–8 as well as a working memory component in trials 6–8. (A) Both groups tended to make fewer errors over trials on day 1 ($p < .10$), and made significantly fewer errors over trials on day 3 ($p < .05$). Therefore both groups appear to perform similarly on trials that assess working memory only. The CORT group made significantly more errors on days 2 and 4 than the VEH group ($p < .05$). It is interesting to note that the VEH animals made fewer errors on 7 of the 8 trials on days 2 and 4 than their best performance from the previous day. In contrast, CORT animals exhibited very slight improvements on only 2 of 8 trials over days 2 and 4 relative to their best performance on previous day's trials. Thus the overnight rest period appeared to be followed by improved performance in the vehicle group only. (B) Time to goal measures supported conclusions drawn from error measures. Both groups took less time to find the goal over trials on day 1 and on day 3. The CORT group took significantly more time to find the goal position on days 2 ($p < .05$) and 4 ($p < .06$) than the VEH group. The VEH group took less time on all trials of days 2 and 4 than on any trial from the previous day, whereas the CORT group did not exhibit such improvements in performance. For both the CORT and VEH groups, the differences in the time to find the goal box corresponded to trials where there was a difference in the number of errors.

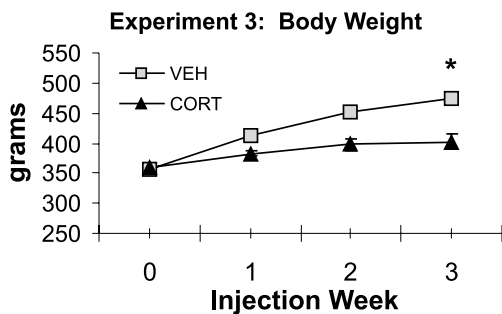


Fig. 3. After 21 days of treatment, the CORT group in Experiment 3 had significantly lower body weight than the VEH group. The significant reduction in weight gain of the CORT group relative to VEH group is consistent with well-known effects of glucocorticoids on body weight, and therefore provides independent confirmation of the effectiveness of the CORT treatment in Experiment 3. * $p < .001$.

Similar to the error measures, the time to goal box was not significantly different across groups on days 1 and 3 [day 1: $F(1, 17) = .88, p > .05$; day 3: $F(1, 17) = 2.42, p > .05$; see Fig. 4B]. Subjects took less time to find the goal box over trials on both days [day 1: $F(4, 68) = 9.513, p < .05$; day 3: $F(4, 68) = 2.94, p < .05$]. There was no group by trial interaction on either day [day 1: $F(4, 68) = 0.333, p > .05$; day 3: $F(4, 68) = 0.414, p > .05$]. On day 2 test trials took place 24 h after introduction of the first goal position. The CORT group took significantly longer to find the goal position [day 2: $F(1, 17) = 6.50, p < .02$], an effect that coincides with the greater number of errors made by this group. There was also an effect of trial [$F(3, 51) = 3.69, p < .05$] and a group by trial interaction [$F(3, 51) = 4.04, p < .05$]. On day 4 test trials also took place 24 h after the introduction of a different goal position. The CORT group tended to take longer [$F(1, 17) = 3.97, p = .06$] than the VEH group, again with the longer time corresponding to the greater number of errors made by the CORT group on day 4. There was no effect of trial [$F(3, 51) = .174, p > .05$] or group by trial interaction [$F(3, 51) = 1.17, p > .05$].

To test whether CORT impaired reference memory, trials that relied on reference memory only (trial 1 of days 2 and 4) were compared to the previous working memory trials (trial 4 of days 1 and 3). Because the previous analyses indicated that memory impairments occurred on both days 2 and 4, but not on days 1 and 3, the corresponding trials of interest were combined to form an average. Errors were analyzed with a repeated measures ANOVA with trial (trial 4 from days 1 and 3 vs. trial 1 from days 2 and 4) as the within subjects factor and group (VEH, CORT) as the between subjects factor. This analysis revealed a main effect of group [$F(1, 17) = 7.04, p < .05$] and an interaction [$F(1, 17) = 5.98, p < .05$, see Fig. 5]. VEH animals had significantly fewer errors on trial 1 of days 2 and 4 than on trial 4 of days 1 and 3 as demonstrated by the post hoc comparisons between these trials [$t(8) = -2.826, p < .05$]. VEH performance may have improved during the overnight delay through consolidation of memories from the previous day's events. CORT animals did not reduce the number of errors on trial 1 of days 2 and 4 relative to the last trial of the previous day as revealed by a contrast comparison [$t(9) = 1.670, p > .05$]. Similar analyses were performed for the time to goal measure. Although the pattern of effects for time to goal position was consistent with findings for errors on these trials, the interaction was not significant, but the VEH group overall performed better than the CORT group [$F(1, 17) = 7.25, p < .05$].

4.2.3. Summary of results

The effectiveness of the CORT dose (26.8 mg/kg body weight) was verified by demonstration of a reduction in

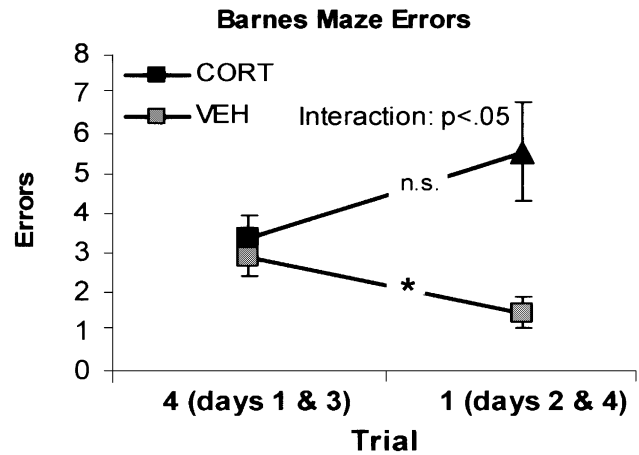


Fig. 5. When comparing number of errors on the last trial of days 1 and 3 to the first trial of days 2 and 4, the latter trials utilizing reference memory selectively, there was an interaction between group and trial. Post hoc contrast comparisons revealed that the control group (VEH) reduced the number of errors on the reference memory trial relative to the last trial from the previous day. In contrast, CORT animals exhibited no such improvement. The data suggest that CORT animals were unable to consolidate memories over the rest period. * $p < .05$.

body weight gain in the CORT group relative to the VEH group. Spatial working memory in the Barnes maze, like the Y-maze, was not affected by 21-day administration of CORT, suggesting that the conclusions from Experiments 1 and 2 can be generalized across tasks. In contrast, reference memory was impaired in the CORT group. These findings suggest that reference memory is impaired after administration of CORT for only 21 days. This conclusion is further supported by the finding that the VEH group improved on trials that utilized reference memory only, whereas the CORT group did not.

5. Discussion

In the first experiment, animals were injected with CORT or VEH or handled, then tested for spatial working memory in the Y-maze. All three groups showed evidence of intact spatial working memory. In experiment 2, the number of subjects per group was increased to ensure sensitivity to spatial working memory deficits, and subjects were tested at three time points: 3 days prior to the start of injections (pre-treatment), after 21 daily injections of CORT or VEH, and again after 56 daily injections. Again, spatial working memory was intact after 21 daily injections, but impairments in spatial working memory were observed in CORT animals after 56 daily injections. The failure to detect spatial working memory deficits after 21 days could have been related to the relative ease of the Y-maze test, so we re-tested 21-day CORT effects on spatial working memory with a more difficult task, the Barnes maze

(Experiment 3). Because rats in this experiment were tested across several days, reference memory was also tested. Again, working memory was not disrupted by 21-day administration of CORT. However, spatial reference memory in the Barnes maze was impaired. This conclusion was based on the findings that impairments were restricted to trials that occurred 24 h following the introduction of each goal position, and were apparent on trials that selectively tested reference memory.

These studies were carried out so that the influence of extraneous factors was minimized. The tasks chosen minimized the influence of CORT-induced weight loss and hypothalamic–pituitary–adrenal axis suppression. Such factors could have been considerable with memory tasks that rely on food and water restriction or exposure to aversive conditions. By using three separate experiments, all testing could start 24 h after the last injection, and avoided transference of maze experience across tasks. In Experiment 2, pre-treatment tests confirmed that there were no group differences prior to treatment. Although animals in Experiment 2 were tested multiple times, the maze was moved to a new room each time to avoid transference of test experience. That strategy appeared to work since the control animals spent proportionally more time in the novel arm during all three tests.

The dose used in this study produced, in a separate group of animals, significant elevations in circulating plasma CORT concentrations ($42.63 \pm 7.22 \mu\text{g}/\text{dl}$) relative to CORT concentrations in VEH. In two separate groups of animals, we found that CORT elevations from exposure to the laboratory on a busy day were similar to the elevations produced by this dose. Thus the concentrations of CORT found with this dose are within the physiological range produced during stress. These plasma concentrations are also comparable to those reported after the first exposure to restraint stress ($30\text{--}46 \mu\text{g}/\text{dl}$; Watanabe et al., 1992). The dose used by Woolley et al. (1990) was 10 mg per day per rat in 250–300 g rats. In our first study, rats arrived in the laboratory between 250 and 300 g, but quickly gained weight over the two weeks of habituation. As a result, the rats weighed more than 250–300 g at the start of the injections so that the dose of CORT used, 10 mg/day, was approximately 26.8 mg/kg body weight for our study. The dose of 26.8 mg/kg body weight was then used for experiments 2 and 3. This dose may be lower than that used by Woolley et al. (1990), if the animals in that study were between 250 and 300 g at the start of injections rather than before habituation to the colony.

The dose of CORT used in the present experiments caused effects in the periphery that are consistent with effects previously seen in animals and in humans. While body weight increased in the VEH group, growth suppression was sustained for as long as 56 days in the CORT group. These findings are consistent with reports

that prescription glucocorticoids that are used to treat asthma in children can, although do not always, reduce growth (Saha, Laippala, & Lenko, 1997). Growth suppression has been reported as transient following chronic multiple stress in rats, but sustained over 21 days of restraint stress (Magarinos & McEwen, 1995). In addition to reduced body weight, the weight of the gastrocnemius muscle, which is known to be vulnerable to prolonged elevation of glucocorticoids, was significantly lower than VEH after both durations of CORT. Gastrocnemius muscle had a lower muscle to body weight ratio after 56 days of CORT, suggesting that this muscle was affected relatively more than can be accounted for by the lower body weight. These findings are consistent with muscle wasting seen in humans undergoing prolonged elevations in cortisol (Hickson & Marone, 1993). In contrast, the soleus muscle is known for resistance to the effects of glucocorticoids, and remained unaffected after 21-day administration of CORT. Surprisingly, the soleus weight was reduced after 56 days of CORT administration, but the soleus to body weight ratio was similar for CORT and VEH so that the soleus weight reduction may have been associated more closely with the reduced weight bearing load. Adrenal gland weights were also lower in CORT animals relative to VEH after both durations of treatment. Although adrenal gland hypertrophy can occur following chronic stress (Magarinos & McEwen, 1995), exogenous CORT administration reduces adrenal gland weight, which likely reflects a compensatory reduction in endogenous CORT release. In pilot animals, we have seen adrenal gland weight reduction as early as 5 days, and here the weight reduction continued after 21 and 56 days of CORT treatment. This organ weight reduction accounts, in part, for the adrenal suppression seen during and following abrupt cessation of cortisol treatment in humans (Kannisto, Korppi, Remes, & Voutilainen, 2000). Adrenal gland weight reduction exceeded the reduction in overall body weight after both 21 and 56 days of treatment. Overall, the CORT effects on organ and muscle weights, along with growth suppression are consistent with previous demonstrations of glucocorticoid effects on organs and tissues, and support the effectiveness of the CORT dose used in the present experiments.

Short durations of synthetic glucocorticoids (dexamethasone and betamethasone) have anabolic effects on heart and increase heart weight (Clark, DeMartino, & Wildenthal, 1986; Clark, Tandler, & Vignos, 1982; Kelly & Goldspink, 1982). In contrast, longer durations can convert cardiac muscle to a catabolic state, and cause weight reduction (Kurowski & Czerwinski, 1990). Although we found CORT animals to have lower heart weight, the heart to body weight ratio did not differ. Although the heart weight differences may reflect the reduced circulatory demands associated with the lower

body weight, it is important to note that CORT-induced cardiac hypertrophy has previously been accompanied by ultrastructural damage (Clark et al., 1982).

CORT administration for 21 days did not reduce behavioral activity in Experiment 1, but did reduce activity in Experiment 2. This reduction is consistent with behavioral changes in open-field exploration reported after 21 days of restraint stress (Conrad et al., 1999). There was no indication in Experiment 3 that CORT caused a reduction in activity, but the differences in testing conditions may have overshadowed such an effect. The inconsistency in the findings from Experiments 2 and 3 highlight the need to determine whether CORT interacts with the novelty and perceived safety of the environment to influence activity levels.

The failure to detect spatial working memory deficits after 21 days of CORT administration was consistent over the three experiments despite a number of differences across the tasks employed. In the Barnes maze, rats must differentiate the goal from many more alternate positions than in the Y-maze. The intertrial interval differs across the two mazes. Rats tested on the Barnes maze are on an open platform in a bright room, whereas rats in the Y-maze are within dark walls in a dimly lit room. Because of the numerous differences between these two mazes, we believe the conclusion that spatial working memory is intact after 21-day administration of CORT is generalizable rather than specifically related to the testing procedures employed.

Although rats receiving CORT for 21 days did not exhibit spatial working memory deficits in the Barnes or Y-maze, it has previously been demonstrated that 21 days of restraint stress causes spatial working memory deficits in two different mazes (Conrad et al., 1996; Luine, Villegas, Martinez, & McEwen, 1994). The duration of CORT treatment required to cause spatial working memory deficits may be longer than the duration required for restraint stress. In support of that assertion, 56 daily injections of CORT in Experiment 2 caused working memory deficits in the Y-maze. These data suggest that longer durations of the CORT dose employed can impair spatial working memory. Taken together, the data suggest that spatial working memory deficits accumulate over longer treatment conditions than are required for restraint stress and CORT-induced reference memory deficits. It seems likely that the response to restraint stress includes sympathetic nervous system activation, and its consequent activation of the adrenal medulla. Perhaps the greater physiological complexity of the stress response accounts for the spatial working memory deficits seen after only 21 days of restraint.

The CORT-related deficits in Barnes maze performance reported here were restricted to test trials that occurred 24 h after the introduction of each goal position, and were not present on test trials that occurred

within 1½ h of the first exposure to each goal position. Deficits related to motivational differences, differences in the willingness to explore, or debilitation may be ruled out since these would be expected to either persist across all days, or subside over testing days. They would not be expected to fluctuate across days as was observed. Perseveration of errors in the Barnes maze would be consistent with age-related performance on spatial memory tasks and corresponding age-related elevations in glucocorticoids. However, there is no clear reason why perseverative errors would be present on days 2 and 4, but not days 1 and 3 of Barnes maze testing. Differences caused by perseveration would be expected to be most noticeable on day 1 when the goal location was first changed relative to the location during habituation, and again on day 3, when the goal position was moved relative to the previous nine trials. The CORT-induced deficits are more likely to be related specifically to the forms of memory used on days 2 and 4.

Because of the lengthy inter-session interval, the trials on days 2 and 4 included a reference memory component. Despite both groups having similar mean numbers of errors on the last test trials of days 1 and 3, visual inspection of the data reveals that the CORT group made more errors on all trials on days 2 and 4, including the first reference memory trial. In contrast, the control group made fewer errors and took less time to find the goal box on trial 1 of days 2 and 4 than on the last trial of the previous day (trial 4 of days 1 and 3). This pattern suggests that the control group, in contrast to the CORT group, consolidated information about the goal position overnight to improve performance on the following day. This pattern of findings held true for both the number of errors, and the corresponding increase in time required to find the goal box. It is also important to note that the impaired rats made the same number of errors in the same amount of time on days 1 and 3, and therefore it is unlikely that deficits on days 2 and 4 can be accounted for by slowed performance, and difficulties related to the failure to gain body and muscle weight. Instead the CORT group failed to consolidate spatial information from the preceding trials.

The mechanisms by which chronic 21-day elevations in CORT impair reference memory, and 56-day elevations in CORT impair working memory, are unknown. The deficits reported are not caused by acutely elevated CORT because the testing commenced 24 h after the last injection. Furthermore the deficits in longer-term memory were not apparent until 2 and 4 days after the last injection. The deficits are likely to be a secondary consequence of chronically elevated CORT. We presume the CORT group underwent hippocampal anatomical alterations, particularly in CA3 (Woolley et al., 1990). Although we have not seen gross structural alterations in the form of cell or volume reduction (Coburn-Litvak et al., 1999), it is likely that ultrastructural

changes are present. It is also possible that the deficits are related to suppression of the hypothalamic–pituitary–adrenal axis. Although this was not measured directly in this study, the dramatic adrenal gland atrophy is highly suggestive of adrenal suppression. The selectivity of the deficits after 21 days of CORT administration to trials that solely or partially test reference memory suggests that alterations caused by chronic CORT administration may interact directly or indirectly with mechanisms of memory consolidation. It seems unlikely that any structural damage in CA3 that occurs as early as 21 days accounts for working memory deficits, since longer CORT elevations were required to impair this form of memory. It is likely, however, that damage in the hippocampus accumulates over the duration of treatment, and that the additional damage after 56 daily injections is more closely associated with the working memory deficits reported here.

The finding that CORT causes memory deficits is consistent with previous reports of spatial learning deficits after CORT treatment for 2 months (Bardgett, Taylor, Csernansky, Newcomer, & Nock, 1994), 80 days (McLay et al., 1998) and 3 months (Bodnoff et al., 1995). Although some studies report memory deficits in aging, but not young animals (Bodnoff et al., 1995), other studies, like the present one, have reported deficits in young animals (Bardgett et al., 1994; McLay et al., 1998). Shorter treatment durations in young animals may yield very different effects than the same durations in old animals. A 15-day treatment in very old rats enhanced spatial learning (Hebda-Bauer, Morano, & Therrien, 1999). In that study, Morris water maze testing occurred over days and would have utilized reference memory. Although the CORT dose (20 mg/kg, yielding plasma concentrations of 35–42 µg/dl) was similar to ours, administration over 15 days did not significantly influence water maze performance of young rats. Using restraint stress, Luine, Martinez, Villegas, Magariños, and McEwen (1996) have found enhanced spatial working memory after 13 days, and deficits in spatial working memory after 21 days. Our data support their conclusion that the effects seen at 21 days do not necessarily generalize to shorter or longer durations of treatment.

These are the first experiments to differentiate impairments in spatial working and reference memory after different durations of CORT treatment. CORT given for 21 and 56 days significantly affected organ and body weight. Even so, CORT given for 21 days did not influence spatial working memory in either of the mazes despite their substantial differences. In contrast, 21-day administration of CORT impaired spatial reference memory. This was seen in the Barnes maze, where the CORT group performed less well on test trials that took place after a 24-h inter-session interval. Analysis of trials reflecting only reference memory revealed that the VEH group made fewer errors relative to the previous work-

ing memory trial, whereas the CORT group showed no such improvement. When CORT elevations were extended to 56 days, deficits in spatial working memory became apparent. In conclusion, chronic CORT administration impairs reference memory before working memory.

Acknowledgments

We thank Rabia Razi, Noriko Kawashima, Dave Adamo, and Lee Shapiro for help with animal handling. We thank Glenn Hudson, and Bob Chorley for construction of the mazes and Bill Guethlein and Ralph Molaro for developing software for automated data collection. This work was supported by the National Institute of Mental Health Grants MH62075 to B.J.A. and F31MH12677 to P.C.-L.

References

- Barnes, C. A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative and Physiological Psychology*, *93*(1), 74–104.
- Bardgett, M. E., Taylor, G. T., Csernansky, J. G., Newcomer, J. W., & Nock, B. (1994). Chronic corticosterone treatment impairs spontaneous alternation behavior in rats. *Behavioral and Neural Biology*, *61*, 186–190.
- Bodnoff, S. R., Humphreys, A. G., Lehman, J. C., Diamond, D. M., Rose, G. M., & Meaney, M. J. (1995). Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. *Journal of Neuroscience*, *15*, 61–69.
- Carroll, B. J., Curtis, G. C., Davies, B. M., Mendels, J., & Sugeran, A. A. (1976). Urinary free cortisol excretion in depression. *Psychological Medicine*, *6*(1), 43–50.
- Chrousos, G. P., & Gold, P. W. (1992). The concepts of stress and stress system disorders: Overview of physical and behavioral homeostasis. *Journal of the American Medical Association*, *267*, 1244–1252.
- Clark, A. F., DeMartino, G. N., & Wildenthal, K. (1986). Effects of glucocorticoid treatment on cardiac protein synthesis and degradation. *American Journal of Physiology*, *250*, C821–C827.
- Clark, A. F., Tandler, B., & Vignos, P. J. (1982). Glucocorticoid-induced alterations in the rabbit heart. *Lab Investigation*, *47*(6), 603–610.
- Coburn-Litvak, P. S., Encarnacion, R., & Anderson, B. J. (1999). Cell density in CA3 is unchanged after 21 days of corticosterone. *Society for Neuroscience Abstracts*, *25*(1), 856.
- Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y-maze, and this effect is blocked by tianeptine pretreatment. *Behavioral Neuroscience*, *110*(6), 1321–1334.
- Conrad, C. D., LeDoux, J. E., Magarinos, A. M., & McEwen, B. S. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioral Neuroscience*, *113*(5), 902–913.
- Dunn, J. D., & Orr, S. E. (1984). Differential plasma corticosterone responses to hippocampal stimulation. *Experimental Brain Research*, *54*, 1–6.
- Frick, K. M., Baxter, M. G., Markowska, A. L., Olton, D. S., & Price, D. L. (1995). Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiology of Aging*, *16*(2), 149–160.

- Hebda-Bauer, E. K., Morano, M. I., & Therrien, B. (1999). Aging and corticosterone injections affect spatial learning in Fischer-344 X Brown Norway rats. *Brain Research*, 827, 93–103.
- Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. *Trends in Neuroscience*, 20(2), 78–84.
- Hickson, R. C., & Marone, J. R. (1993). Exercise and inhibition of glucocorticoid-induced muscle atrophy. *Exercise and Sports Science Reviews*, 21, 135–167.
- Jarrard, L. E. (1995). What does the hippocampus really do? *Behavioral Brain Research*, 71(1–2), 1–10.
- Kannisto, S., Korppi, M., Remes, K., & Voutilainen, R. (2000). Adrenal suppression, evaluated by a low dose adrenocorticotropin test, and growth in asthmatic children treated with inhaled steroids. *Journal of Clinical Endocrinology and Metabolism*, 85(2), 652–657.
- Kelly, F. J., & Goldspink, D. F. (1982). The differing responses of four muscle types to dexamethasone treatment in the rat. *Biochemical Journal*, 208(1), 147–151.
- Kurowski, T. T., & Czerwinski, S. M. (1990). Glucocorticoid modulation of cardiac mass and protein. *Medicine and Science in Sports and Exercise*, 122(3), 312–315.
- Luine, V., Martinez, C., Villegas, M., Magariños, A. M., & McEwen, B. S. (1996). Restraint stress reversibly enhances spatial memory performance. *Physiology and Behavior*, 59(1), 27–32.
- Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research*, 639, 167–170.
- Magarinos, A. M., & McEwen, B. S. (1995). Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Comparison of stressors. *Neuroscience*, 69(1), 83–88.
- McLay, R. N., Freeman, S. M., & Zadina, J. E. (1998). Chronic corticosterone impairs memory performance in the Barnes Maze. *Physiology and Behavior*, 63(5), 933–937.
- McLay, R. N., Freeman, S. M., Harlan, R. E., Kastin, A. J., & Zadina, J. E. (1999). Tests used to assess the cognitive abilities of aged rats: Their relation to each other and to hippocampal morphology and neurotrophin expression. *Gerontology*, 45(3), 143–155.
- Rubinow, D. R., Post, R. M., Savard, R., & Gold, P. W. (1984). Cortisol hypersecretion and cognitive impairment in depression. *Archives of General Psychiatry*, 41(3), 279–283.
- Ruzek, M. C., Pearce, B. D., Miller, A. H., & Biron, C. A. (1999). Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. *Journal of Immunology*, 162, 3527–3533.
- Saha, M. T., Laippala, P., & Lenko, H. L. (1997). Growth of asthmatic children is slower during than before treatment with inhaled glucocorticoids. *Acta Paediatrica*, 86, 138–142.
- Seed, J. A., Dixon, R. A., McCluskey, S. E., & Young, A. H. (2000). Basal activity of the hypothalamic-pituitary-adrenal axis and cognitive function in anorexia nervosa. *European Archives Psychiatry Clinical Neuroscience*, 250, 11–15.
- Sheline, Y. I., Sanghavi, M., Mintun, M. A., & Gado, M. H. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *Journal of Neuroscience*, 19(12), 5034–5043.
- Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G., & Vannier, M. W. (1996). Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences*, 93, 3908–3913.
- Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F. X., & Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience*, 97, 253–266.
- Starkman, M. N., Gebarski, S. S., Berent, S., & Schteingart, D. E. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biological Psychiatry*, 32(9), 756–765.
- Starkman, M. N., Giordani, B., Berent, S., Schork, M. A., & Schteingart, D. E. (2001). Elevated cortisol levels in Cushing's disease are associated with cognitive decrements. *Psychosomatic Medicine*, 63(6), 985–993.
- Starkman, M. N., Giordani, B., Gebarski, S. S., Berent, S., Schork, M. A., & Schteingart, D. E. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry*, 46, 1595–1602.
- Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research*, 588, 341–345.
- Woolley, C. S., Gould, E., & McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research*, 531, 225–231.