

Prolonged Moderate Elevation of Corticosterone Does Not Affect Hippocampal Anatomy or Cell Proliferation Rates in Mountain Chickadees (*Poecile gambeli*)

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ABSTRACT: Chronic stress and corresponding chronic elevations of glucocorticoid hormones have been widely assumed to have deleterious effects on brain anatomy and functions such as learning and memory. In particular, it has been suggested that chronic elevations of glucocorticoid hormones result in death of hippocampal neurons and in reduced rates of hippocampal neurogenesis. It is not clear, however, if any increase in glucocorticoid levels has negative effects on hippocampal anatomy as many animals regularly maintain moderately elevated levels of glucocorticoids over long periods of time under natural energetically demanding conditions. We used unbiased stereological methods to investigate whether mountain chickadees (*Poecile gambeli*) implanted for 49 days with continuous time-release corticosterone pellets, designed to approximately double the baseline corticosterone levels, differed from placebo-implanted chickadees in their hippocampal anatomy

and cell proliferation rates. We found no significant differences between corticosterone and placebo-implanted birds in either telencephalon volume, volume of the hippocampal formation, or the total number of hippocampal neurons. Cell proliferation rates, measured as the total number of BrdU-labeled cells in the ventricular zone adjacent either to the hippocampus or to the mesopallium, were also not significantly different between corticosterone and placebo-implanted chickadees. Our results suggest that prolonged moderate elevation of corticosterone might not provide the suggested deleterious effects on hippocampal anatomy and neurogenesis in food-caching birds and, as we have shown previously, it actually enhances spatial memory. © 2004

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INTRODUCTION

It has been generally assumed that chronic stress and associated prolonged chronic elevation of glucocorticoid hormones have negative effects on cognitive

abilities of animals such as learning and memory by causing neuronal death and reducing neurogenesis (Sapolsky, 1992, 1996; McEwen and Sapolsky, 1995; McEwen, 2000). Most research on the effects of prolonged stress and prolonged elevation of glucocorticoid hormones on memory and the brain has been done on mammals and focused specifically on the hippocampus, the structure thought to be responsible for memory processing (McEwen and Sapolsky, 1995; Sousa et al., 1998; Gould and Tanapat, 1999; Leverenz et al., 1999; Ohl et al., 1999; Lucassen et al., 2001). It has been suggested that prolonged chronic

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elevation of glucocorticoid hormones should result in structural differences in the hippocampus such as reduced volume, fewer hippocampal neurons and reduced neurogenesis (McEwen and Sapolsky, 1995; Ohl et al., 1999; Gould and Tanapat, 1999). Recently, however, the concept that long-term elevation of glucocorticoids should always result in hippocampal damage has been challenged (Sousa et al., 1998; Laverenz et al., 1999).

In birds, the hippocampal formation has also been proposed to be responsible for spatial memory processing (Krebs et al., 1989, 1996), yet little is known about the effects of prolonged chronic stress on the avian hippocampus. Birds respond to unpredictable changes in their social and physical environments by elevating corticosterone levels above baseline (Wingfield et al., 1997, 1998; Silverin, 1998). Whereas short-term elevations (generally hours or days) have been consistently regarded as beneficial for learning and memory, prolonged elevations of glucocorticoid hormones (generally weeks or months) are thought to be deleterious for both birds and mammals (McEwen and Sapolsky, 1995; Wingfield et al., 1997, 1998).

Most studies describing negative effects of prolonged elevation in glucocorticoid hormones have used fairly large, stress-induced levels of such hormones; however, little is known about moderate elevations, which exceed baseline by only two- or threefold (Pravosudov, 2003). Such moderate elevations are considerably lower than stress-induced levels, which could exceed baseline by more than 600% (e.g., Pravosudov et al., 2001, 2003). For example, free-ranging willow tits (*Parus montanus*), small food-caching birds, seem to experience seasonal variation in baseline corticosterone levels with the highest levels maintained over several winter months when foraging conditions are most demanding (Silverin, 1998). These winter baseline corticosterone levels, however, appeared only moderately (two–threefold) higher than baseline levels during the rest of the year, and they were much lower than acute stress-induced levels (Silverin, 1998). Food-caching birds hide thousands of food items throughout their home range and then retrieve them during energetically challenging times during winter (Pravosudov and Grubb, 1997). They rely, at least in part, on spatial memory to retrieve their caches, and thus spatial memory is an important fitness component for these birds because successful cache retrieval could be crucial for survival (Krebs et al., 1996). Because cache retrieval is most critical during the winter when baseline corticosterone levels are likely to be moderately elevated due to the unpredictable environment (Silverin, 1998; Pravosudov et al., 2001), it is important to understand the

effect of these elevated glucocorticoids on spatial memory and the hippocampus in food-caching birds.

Previously, Pravosudov et al. (2001) demonstrated that a long-term unpredictable food supply resulted in moderate (approximately twofold, compared to 600% or greater elevations in response to acute stress, Pravosudov et al., 2001, 2003) but significant elevation of baseline corticosterone levels in mountain chickadees (*Poecile gambeli*) in the laboratory, and that these birds also demonstrated enhanced spatial memory (Pravosudov and Clayton, 2001). In a first, behavioral part of this study, Pravosudov (2003) implanted mountain chickadees with corticosterone and demonstrated that moderate elevation of corticosterone (approximately 140% above baseline) for 49 days indeed enhances spatial memory. Thus, it appears that in food-caching birds, which reside permanently on territories throughout the year, even prolonged moderate elevation of corticosterone in response to unpredictable environmental conditions may serve as an adaptation by enhancing spatial memory that is crucial for retrieving cached food (Pravosudov, 2003). An important question, however, remains about the effect of prolonged moderate elevation of corticosterone on the hippocampus, the brain structure responsible for spatial memory processing. Whereas it is widely assumed that any chronic elevation in glucocorticoids should have deleterious effects on memory and the brain, our previous results suggest that prolonged moderate elevation of corticosterone may not have a negative impact on the hippocampal formation of food-caching mountain chickadees as their spatial memory was enhanced by such treatment (Pravosudov, 2003).

In this part of the study, we investigated the brains of the mountain chickadees used in a previous behavioral experiment in which they were implanted with continuous release corticosterone pellets for 49 days and showed enhanced spatial memory performance compared to placebo-implanted birds (Pravosudov, 2003). Mammalian studies declaring effects of prolonged chronic stress on memory and the brain used different amount of time to quantify chronic stress, with most studies considering period of 21–56 days long enough (Bartolomucci et al., 2002; Bowman et al., 2003; Coburn et al., 2003; Conrad et al., 2003). Some studies, on the other hand, used much longer periods of time to quantify the effects of long-term stress (Laverenz et al., 1999; Sousa et al., 1998). The time period used in our study (49 days) falls within the accepted range for prolonged chronic effects, and it also seems to reflect rather long-term effects for a small bird like the mountain chickadee with a relatively short life expectancy (1.5–2 years). Using unbiased stereological methods, we specifically tested

whether prolonged moderate elevation of corticosterone had an effect on (a) hippocampal volume, (b) total number of hippocampal neurons, and (c) hippocampal cell proliferation rates.

METHODS

Twenty-eight mountain chickadees were caught around Sage Hen, Tahoe National Forest, CA, between October 13–16, 2002, using mist nets near baited feeders. All birds were transported to the laboratory at the University of California in Davis, placed individually in wire-mesh cages (60 × 42 × 60 cm) and maintained on a 9:15-h light:dark cycle at a constant 20°C for the duration of the experiment. Birds were fed with a mixture of pine nuts, shelled and unshelled sunflower seeds, crushed peanuts, and mealworms, and given water with vitamins *ad libitum*.

First, corticosterone implants were calibrated in a subset of four mountain chickadees, which consequently were not used in the behavioral experiments (Pravosudov, 2003). Birds received implants of different size (0.01, 0.025, 0.05, and 0.1 mg of corticosterone) on November 1, 2002, and within 2 weeks baseline plasma corticosterone increased from 21% (0.01 mg implant) to 186% (0.1 mg implant) over preimplant baseline (Pravosudov, 2003). There was a highly significant positive correlation between the implant size and the amount of corticosterone elevation ($R^2 = 0.99$; Pravosudov, 2003).

On December 5, 2002, 12 mountain chickadees (eight males and four females, determined after birds were sacrificed for brain analyses) were implanted with commercially available biodegradable 90-day continuous time release pellets (Innovative Research of America) containing 0.075 mg of corticosterone (which are reported to release approximately 833 ng of corticosterone per day over 90 days) and 12 chickadees (seven males and five females) were implanted with matched placebo pellets. All pellets were 1.5 mm in diameter; they were placed subcutaneously in the flank. For the experiment, we chose implants containing 0.075 mg of corticosterone because based on our calibration they should result in approximately a 140% increase in baseline corticosterone levels (Pravosudov, 2003), which imitates the natural elevations (Silverin, 1998) and elevations resulting from unpredictable food supplies found in Pravosudov et al. (2001). This is a moderate increase, as mountain chickadees typically show >600% increases above baseline during a standardized response to acute stress (Pravosudov et al., 2001, 2002, 2003).

Starting at the 15th day after implantation, birds were tested individually in a cache recovery task and subsequently in two versions of a one-trial associative learning task between December 12, 2002 and January 22, 2003 (Pravosudov, 2003). The last behavioral test ended on the 48th day after implantation. After behavioral testing, all birds were injected with a 75 mg/kg 5-bromo-2'-deoxyuridine (BrdU) solution into the breast muscle once on January 22 and the second time on January 23, and then sacrificed

for the brain analyses 1 day later. All birds spent 49 days with corticosterone implants before the BrdU injections.

Birds were anesthetized (0.03 mL per bird of 50 mg/mL Nembutal–sodium solution) and perfused transcardially with 100 mL of phosphate buffer (0.1 M PO_4) followed by 100 mL of 4% paraformaldehyde in phosphate buffer. After perfusion, birds were decapitated and their brain (within the skull) was placed in 4% paraformaldehyde for 1 week. We then removed the brains from the skull and postfixed them in 4% paraformaldehyde for an additional week, after which all brains were cryoprotected in a 30% sucrose solution, frozen, and kept at -20° until processing. We cut coronal sections at 40 μm on a sliding, freezing microtome, and collected every section in phosphate-buffered saline (PBS, 0.1 M, pH 7.4). Every fourth section was mounted onto gelatin-coated slides, Nissl-stained with thionin, and coverslipped with Permount for measurements of the hippocampal formation volume and the total number of hippocampal neurons. Sections for BrdU immunohistochemistry (also every fourth section) were stored at -20° in a cryoprotectant solution until processing.

Volumetric Measurements and Neuron Counts

We used StereoInvestigator software (version 3.15a, Microbrightfield, Colchester, VT) for all stereological measurements. To measure the volume of the hippocampal formation and the telencephalon on Nissl-stained slides, we used the Cavalieri principle (Gundersen and Jensen, 1987; West and Gundersen, 1990), employed successfully before with chickadees (Pravosudov and Clayton, 2002; Pravosudov et al., 2002). We determined the boundaries of the hippocampal formation as described in Krebs et al. (1989), and we used a total of 13 to 18 sections per bird for hippocampal measurements (480 μm apart, 1 in 12 sections). The first section was chosen randomly from the first four sections containing the hippocampus, according to the Cavalieri principle (Gundersen and Jensen, 1987; West and Gundersen, 1990), and we used a 200- μm grid size. This sampling method has proven to be most efficient in chickadees by providing a low variance of individual estimates (Pravosudov and Clayton, 2002; Pravosudov et al., 2002). We used a total of 15 to 18 sections (640 μm apart, 1 in 16 sections), with the first section chosen randomly from the first four sections and a 1142.86- μm grid size to measure the volume of the telencephalon. All sections were coded prior to the analyses, so all measurements were performed blind with respect to bird identity and experimental design.

To calculate the total number of hippocampal neurons we used the optical fractionator method (West et al., 1991), which combines the fractionator (multistage sampling scheme) with the optical dissector to allow for unbiased counting of neurons (Sousa et al., 1998), on the same Nissl-stained sections that were used for the volumetric measurements. This method allows estimation of the absolute number of neurons independently from the estimates for the hippocampal volume (West et al., 1991). In our

analyses, we used a $900 \mu\text{m}^2$ frame area and $62,500 \mu\text{m}^2$ step area. We used a $\times 100$ Neofluar oil objective on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator system. We used the right half of the hippocampal formation to estimate the number of neurons and then doubled that number to get the total number of hippocampal neurons because there were no significant size differences between the right and left sides of the hippocampal formation (Sign test, $z = 0.20$, $p = 0.84$).

To evaluate the precision of our sampling scheme, we calculated coefficients of error for both volumetric and neuron count measurements. We calculated the relative variance of individual estimates, which allows evaluating the robustness of our sampling scheme (CE; Gundersen and Jensen, 1987; West et al., 1996). The variance of estimates was low for telencephalon volume (mean CE = 0.01, range 0.01–0.02), hippocampal volume (mean CE = 0.02, range 0.01–0.03), and neuron counts (mean CE = 0.06, range 0.04–0.08), which supports the reliability of our measurements and our sampling scheme.

BrdU Labeling

We followed the procedure described in Lavenex et al. (2000) and in Pravosudov and Omanska (2004). We used free-floating sections at room temperature. We first rinsed the sections in 0.1 M PBS (4×10 min), then incubated in 2 N HCl for 1 h, rinsed in 0.1 M borate buffer (pH 8.5) for 15 min, then in PBS (2×10 min), and finally incubated in $1 \mu\text{g}/\text{mL}$ proteinase K in PBS for 30 min. All sections were then washed in PBS (3×5 min), placed in 0.5% hydrogen peroxide in PBS for 30 min, washed again in PBS (3×5 min), and incubated in a blocking solution of 0.1 M PBS with 1 mg/mL bovine serum albumin (BSA), IgG (secondary antibody, Vectastain ABC Kit, Vector Laboratories), and 0.3% Triton X-100 for 1 h. We then incubated all sections overnight in primary anti-BrdU antibody (Beckton Dickinson; 1:500 in PBS/BSA/Triton), rinsed them in PBS (3×5 min), and incubated them in antimouse biotinylated secondary antibody (Vectastain ABC kit) in 0.1 M PBS/BSA/Triton. All sections were then washed in PBS (3×5 min), incubated in ABC reagent (Vectastain ABC kit) in PBS/BSA/Triton, rinsed in PBS (3×5 min), and treated for 10 min in 0.5 mg/mL 3,3'-diaminobenzidine mixed in 0.05 M Tris buffer, pH 7.4 with 0.01% H_2O_2 . Finally, all sections were washed in PBS (3×5 min), Nissl stained in thionin for 1.5 min, mounted on gelatin-coated slides, and coverslipped with Permount.

We counted BrdU-labeled cells in the ventricular zone (VZ) adjacent to the hippocampus (HP) and mesopallium (M, formerly hyperstriatum ventrale) following Patel et al. (1997). All labeled cells were counted on both sides of the hippocampus throughout the entire thickness of the section on every 12th section ($480 \mu\text{m}$ apart) using a $\times 100$ Neofluar oil objective on a Nikon Optiphot microscope linked to the PC-based StereoInvestigator system. The number of BrdU-labeled cells counted in the selected sections

was multiplied by 12 (inverse of the sampling fraction) to get the total number of BrdU-labeled cells.

Statistical Analyses

We used analyses of variance and covariance for all brain data. All data (raw or log-transformed) met the assumptions for parametric statistical procedures. For analyses of relative hippocampal volume, total number of hippocampal neurons, and number of BrdU-labeled cells, we used as a covariate the volume of the telencephalon without the hippocampal formation. We used a power analysis to calculate the power of nonsignificant tests to detect differences that could be expected based on previously published reports using similar time scale (Barnea and Nottebohm, 1994; Smulders et al., 1995, 2000; Pravosudov and Omanska, 2004). For example, Smulders et al. (1995, 2000) reported that hippocampal volume in fully grown food-caching black-capped chickadees (*Poecile atricapillus*) changed by ca. 30% over a 2-month period, and the number of hippocampal neurons increased by ca. 45% from June to October and then decreased by ca. 26% from October to December. Barnea and Nottebohm (1994) reported a 49% increase in neuronal incorporation rates in adult black-capped chickadees from August to October and then a 72% reduction from October to February. Barnea and Nottebohm (1994) measured only neuron incorporation rates, and thus the changes in cell proliferation rates may or may not follow the same trend as neuron incorporation consists of cell proliferation and neuron survival. Previously, we have also found around 40% reduction in cell proliferation rates in subordinate chickadees after 57 days of social interactions (Pravosudov and Omanska, 2004). Thus, we feel it is reasonable to expect a ca. 30% change in hippocampal volume and neuron numbers and a ca. 50% change in cell proliferation rates as a result of our 49-day hormone manipulations, assuming that such manipulations indeed have a significant effect on the hippocampus. Because of the limited number of studies, however, which were able to detect significant differences in the avian hippocampus in adult birds, we also presented statistical power to detect smaller changes and 95% confidence intervals.

RESULTS

There were no significant differences between corticosterone and placebo-implanted chickadees in either wing length, $t(22) = 0.12$, $p = 0.91$, or body mass, $t(22) = -0.74$, $p = 0.46$, at the time the birds were sacrificed (Table 1, Pravosudov, 2003).

There were no significant differences between males and females in any of the measured brain parameters [telencephalon volume, $F(1, 20) = 1.73$, $p = 0.20$; relative hippocampal volume, $F(1, 19) = 0.12$, $p = 0.73$; the total number of hippocampal neurons, $F(1, 19) = 0.14$, $p = 0.71$; number of

Table 1 Body Size and Measured Brain Parameters in Corticosterone (CORT) and Placebo-Implanted Mountain Chickadees

Parameter	Cort	Placebo
Body mass, g	12.1 ± 0.3 (12)	12.2 ± 0.4 (12)
Wing length, mm	70.7 ± 0.7 (12)	70.6 ± 0.7 (12)
Telencephalon volume, mm ³	367.5 ± 39.1 (12)	383.7 ± 43.5 (12)
Hippocampus volume, mm ³	16.7 ± 1.8 (12)	16.8 ± 2.3 (12)
Total number of neurons	1,908,705 ± 63,241 (12)	1,924,559 ± 88,129 (12)
Total number of BrdU-labeled cells in hippocampus (HP) ventricular zone	2,074 ± 547 (7)	2,641 ± 538 (8)
Total number of BrdU-labeled cells in mesopallium (M) ventricular zone	2,988 ± 920 (7)	4,009 ± 1150 (8)

Presented are means, SE and sample size (in parentheses).

BrdU-labeled cells in the ventricular zone adjacent to the hippocampus, $F(1, 10) = 2.55$, $p = 0.18$; number of BrdU-labeled cells in the ventricular zone adjacent to the mesopallium, $F(1, 10) = 2.59$, $p = 0.14$], so we dropped sex from all further analyses.

Corticosterone-implanted birds did not differ from placebo-implanted birds in the volume of telencephalon minus the hippocampal formation (Table 1), $F(1, 22) = 0.92$, $p = 0.35$, or in the absolute volume of the hippocampal formation (Table 1, Fig. 1), $F(1, 22) = 0.02$, $p = 0.90$. Relative volume of the hippocampal formation (controlled for the volume of the rest of the telencephalon) was also not significantly different between corticosterone and placebo-implanted chickadees, $F(1, 21) = 0.01$, $p = 0.97$. Statistical power to detect a 30% difference between the groups in the hippocampal volume was 0.99 and statistical power to

detect 10% hippocampal volume reduction in implanted birds was 0.64.

There were no significant differences in the total number of the hippocampal neurons between corticosterone and placebo-implanted birds (Table 1, Fig. 2) $F(1, 21) = 0.12$, $p = 0.73$; statistical power to detect a 30% difference between the groups was 0.99. Statistical power to detect a 20% reduction in neuron numbers was 0.96, and a 10% reduction—0.53. Similarly, corticosterone-implanted birds did not differ significantly from placebo-implanted birds in the total number of BrdU-labeled cells in the ventricular zone adjacent either to the hippocampus (Table 1, Fig. 3), $F(1, 12) = 0.37$, $p = 0.55$, or to the mesopallium (Table 1, Fig. 3), $F(1, 12) = 0.30$, $p = 0.59$. Statistical power to detect a 50% difference between the groups in cell proliferation rates was 0.40, and statistical power to detect a 30% decrease in hippocampal cell

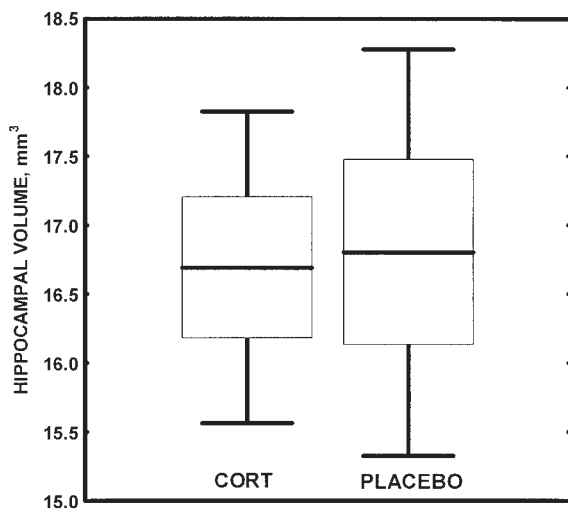


Figure 1 Hippocampal volume of corticosterone-implanted (CORT) and placebo-implanted mountain chickadees. Bars represent S.E. and whiskers represent 95% confidence interval.

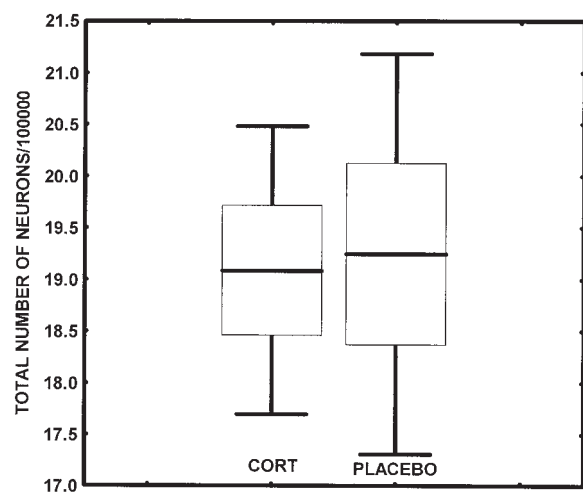


Figure 2 Total number of hippocampal neurons in corticosterone-implanted (CORT) and placebo-implanted mountain chickadees. Bars represent S.E. and whiskers represent 95% confidence interval.

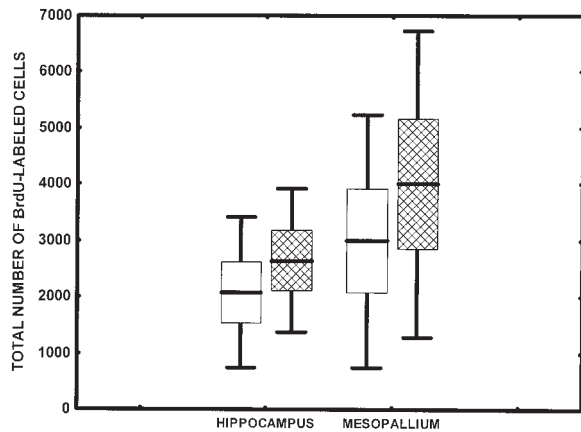


Figure 3 Total number of BrdU-labeled cells in the ventricular zone adjacent to the hippocampus and to the mesopallium in corticosterone-implanted (empty bars) and placebo-implanted (hatched bars) mountain chickadees. Bars represent S.E. and whiskers represent 95% confidence interval.

proliferation rates in corticosterone-implanted birds was 0.25.

We also analyzed cell proliferation rates, hippocampal volume, and the total number of neurons in relation to individual birds memory performance presented in Pravosudov (2003) across both groups. There was no significant relationship between spatial memory performance on a cache-retrieval task (measured as the number of sites inspected to find previously made caches) or on a one-trial associative learning task (measured as the number of sites inspected to find the site that contained food) and either cell proliferation rates in the ventricular zone adjacent to the hippocampus [linear regression, $t(10) = 1.49$, $p = 0.16$, for cache-retrieval performance; $t(10) = 0.31$, $p = 0.76$, for one-trial task performance], hippocampal volume [linear regression, $t(17) = -0.91$, $p = 0.38$, for cache-retrieval performance; $t(19) = -0.28$, $p = 0.78$, for one-trial task performance] or the total number of hippocampal neurons [linear regression, $t(17) = -0.25$, $p = 0.80$, for cache-retrieval performance; $t(19) = 0.77$, $p = 0.45$, for one-trial task performance].

DISCUSSION

Our study demonstrated that prolonged moderate elevation of corticosterone did not result in significant changes in (a) hippocampal volume, (b) total number of hippocampal neurons, or (c) cell proliferation rates in the ventricular zone adjacent to either the hip-

pocampus or mesopallium. First, it is important to know whether corticosterone implants indeed worked in experimental birds. There is no doubt, in our opinion, that implants were working as expected because of several reasons: (1) we have demonstrated that these implants elevate corticosterone levels in a subset of mountain chickadees used for implant calibration (Pravosudov, 2003), and most importantly (2) corticosterone-implanted birds used in this experiment showed significant differences in food consumption rates and in the amount of fat accumulation, which are mediated by corticosterone (Wingfield et al., 1997, 1998; McEwen, 2002; Kitaysky et al., 2003) as well as in food caching rates and in all performed spatial memory tests (Pravosudov, 2003) compared to placebo-implanted birds. Thus, we feel confident that our results demonstrated effects of elevated corticosterone, and such effects were clearly indicated by large behavioral differences between corticosterone and placebo-implanted chickadees.

Our data contradict the hypothesis that prolonged exposure to elevated levels of glucocorticoids should result in reduced hippocampal volume with fewer neurons, and support our previous indirect studies, which showed that long-term unpredictable food supply causes spatial memory enhancement and moderate elevation of corticosterone, but no changes in hippocampal volume or neuron numbers (Pravosudov and Clayton, 2001; Pravosudov et al., 2001, 2002). Low variance of our estimates combined with reasonable statistical power confirms the robustness of our results, minimizing the possibility that we did not have sufficient sample sizes. Such results were expected, however, based on our behavioral results showing that prolonged moderately elevated corticosterone enhances spatial memory in mountain chickadees (Pravosudov, 2003), and they were also in line with some mammalian studies showing no effect of elevated glucocorticoids on the number of neurons in adult animals (Leverenz et al., 1999; Sousa et al., 1998). Sousa et al. (1998) found that long-term (180 days) chronic elevation of corticosterone in neonatal rats results in significant reduction of neuron numbers, but 30- and 90-day chronic corticosterone elevation in adult rats had no significant effect on neuron numbers in the hippocampus. At the same time, Sousa et al. (1998) reported that 30-day corticosterone elevation in adult rats resulted in small volume reduction in some hippocampus layers (hilus of the dentate gyrus and stratum radiatum of the CA3 hippocampal field), and 90-day long corticosterone elevation resulted in larger volumetric reduction of more layers (molecular layer and hilus of the dentate gyrus, stratum oriens, and radiatum of the CA3 hippocampal field).

Leverenz et al. (1999), on the other hand, reported that 12-month cortisol elevation in pigtail macaques (*Macaca nemestrina*) did not affect hippocampal volume and neuron numbers. Leverenz et al. (1999) concluded that chronically elevation of cortisol without stress does not have deleterious effects on the mammalian hippocampus. Our study showed that prolonged moderate elevation of glucocorticoids (49 days) has no significant effect on hippocampal anatomy while providing important benefits of enhanced spatial memory (e.g., Pravosudov, 2003). It remains possible that larger glucocorticoids elevations and/or longer exposure to such elevations could have a deleterious impact on hippocampal structure.

It is possible that the moderate elevations simulated in our study have other yet unknown negative effects on the hippocampus. Whether or not such effects exist, they did not adversely affect spatial memory as corticosterone-implanted birds actually demonstrated enhanced memory performance (Pravosudov, 2003). Because birds do not always maintain moderately elevated corticosterone levels despite its benefits for spatial memory, it is likely that prolonged elevation of corticosterone has other costs. Suppressed immune response may be one of these costs (Wingfield et al., 1997, 1998).

Mammalian studies have demonstrated repeatedly that stress has a negative effect on hippocampal neurogenesis, including both cell proliferation and neuron survival (Gould and Tanapat, 1999; Fuch et al., 2001; Tanapat et al., 2001; Czeh et al., 2002). For example, Tanapat et al. (2001) showed that a simple exposure to a predator's odor caused a reduction in neurogenesis rates in rats. Stress in animals is usually associated with elevated levels of glucocorticoid hormones, and it is assumed that elevated glucocorticoids directly impact neurogenesis (Gould and Tanapat, 1999; Tanapat et al., 2001). Our results show that prolonged but moderate elevation of corticosterone has no significant impact on cell proliferation rates in mountain chickadees, although the statistical power of our tests was fairly low. It remains possible that neuron survival rates could still be affected by our treatment, which would support most of mammalian studies.

We used only two low-dose (75 mg/kg) BrdU injections in our study although Gould and Cross (2002) suggested that such low doses might label only a fraction of the dividing cells, and that higher doses are necessary to label all dividing cells. On the other hand, Rakic (2002) suggested that high and multiple doses of BrdU might produce numerous artifacts and result in overestimation of proliferating cell numbers. There are also some indications that high BrdU doses could be detrimental to the animals' health (Drapeau

et al., 2003). Even if our study underestimated the total number of proliferating cells, we used the same low BrdU dose for both groups of birds, and thus our results should provide a relative comparison between them. It remains possible that elevated corticosterone affected the rate of BrdU uptake (Gould and Cross, 2002), which could also bias our results. Gould and Cross (2002) argued that many factors including stress and hormone manipulations could change the blood flow or blood-brain barrier permeability, and thus could affect BrdU uptake.

Our study provides further confirmation that variation in spatial memory does not have to be associated with changes in hippocampal anatomy or cell proliferation rates (Pravosudov et al., 2002). Birds implanted with corticosterone demonstrated enhanced spatial memory compared to placebo-implanted birds (Pravosudov, 2003), yet these two groups did not differ either in hippocampal volume, total neuron numbers, or cell proliferation rates. Individual differences in spatial memory performance were also unrelated to individual differences in hippocampal volume, total number of neurons, or cell proliferation rates in the ventricular zone.

Whereas larger hippocampal volumes with more neurons have been linked with better spatial memory across different species or populations (Krebs et al., 1996; Pravosudov and Clayton, 2002), there is no evidence that temporary, short-term changes in memory are also accompanied by such changes in the hippocampus on the individual level. There is only one study suggesting that hippocampal volume and neuron numbers change within the same individuals on a short-term basis seasonally (Smulders et al., 1995, 2000). However, these authors had no data on whether memory also changed seasonally, and thus could not demonstrate that changes in memory were accompanied by changes in hippocampal volume or neuron numbers. In contrast, at least two other studies performed on the same species, the black-capped chickadee (*Poecile atricapillus*), as in Smulders et al. (1995, 2000) studies, failed to support the notion that hippocampal volume and neuron numbers indeed change seasonally (Barnea and Nottebohm, 1994; Hoshoooley and Sherry, 2004).

Neurogenesis rates, on the other hand, including both cell proliferation rates and neuron survival rates, have been shown to correlate with changes in spatial memory performance within the same individuals (Gould et al., 1999; Drapeau et al., 2003; Pravosudov and Omanska, 2004), and neurogenesis has been suggested to play a role in memory function (Barnea and Nottebohm, 1994; Gould et al., 1999; Kempermann, 2002; Nottebohm, 2002). Neurogenesis consists of

both cell proliferation and neuron survival (Prickaerts et al., 2004), and it is often not clear which of these processes contribute to the differences reported. Higher rates of neuron incorporation demonstrated by Barnea and Nottebohm (2002), for instance, could have resulted from either higher cell proliferation rates or higher neuron survival rates or both. In our study, we only considered cell proliferation rates. We demonstrated that spatial memory performance might change without changes in cell proliferation rates, suggesting that other mechanisms play a role in mediating corticosterone-induced variation in memory. Whereas statistical power to detect differences in cell proliferation rates between corticosterone and placebo-implanted birds was rather low, we also found no relationship between individual levels of cell proliferation rates and spatial memory performance. This finding reinforces our conclusion that detected changes in spatial memory were not related to changes in cell proliferation rates. In addition, cell proliferation rates in corticosterone-implanted birds tended to be lower in comparison to placebo-implanted chickadees, yet they performed better on spatial memory tests (Pravosudov, 2003). It still remains possible, however, that, unlike cell proliferation rates, neuron survival rates were related to variation in spatial memory in our experiment. Interestingly, Bartolomucci et al. (2002) demonstrated that tree shrews (*Tupaia belangeri*) exposed to chronic psychosocial stress had significantly reduced hippocampal cell proliferation rates yet they showed enhanced spatial memory performance. Such results also suggest that higher cell proliferation rates are not always associated with enhanced spatial memory. Bartolomucci et al. (2002) measured only cell proliferation rates, and thus the reported results do not necessarily contradict the hypothesis that reduced neurogenesis should result in impaired memory performance as neuron survival rates remains unknown.

Diamond et al. (1992) found that moderately elevated corticosterone levels increase the firing rate of hippocampal neurons in rats, which could subserve memory enhancement. Thus, moderately elevated corticosterone could increase the efficiency of existing neurons without affecting the hippocampal structure or neurogenesis in experimental birds. It remains possible that longer exposure to elevated corticosterone would have eventually resulted in neuronal damage and death. However, 49 days do not seem to be long enough to trigger such deleterious effects in mountain chickadees.

In conclusion, our study contradicts the hypothesis that prolonged elevation of glucocorticoid hormones should have deleterious effects on hippocampal anat-

omy and cell proliferation rates in the ventricular zone. The results indicate that prolonged but moderate elevations in corticosterone commonly experienced by animals under natural conditions do not have a significant impact on hippocampal volume, total number of hippocampal neurons, or hippocampal cell proliferation rates, and that they actually enhance spatial memory in a food-caching bird. It remains to be established whether elevated corticosterone levels over longer periods might have deleterious effects, and whether there are other costs associated with maintaining elevated levels of glucocorticoids over long periods of time.

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REFERENCES

- Barnea A, Nottebohm F. 1994. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc Natl Acad Sci USA* 91:11217–11221.
- Bartolomucci A, de Biurrun G, Czeh B, van Kampen M, Fuchs E. 2002. Selective enhancement of spatial learning under chronic psychosocial stress. *Eur J Neurosci* 15: 1863–1866.
- Bowman RE, Beck KD, Luine VN. 2003. Chronic stress effect on memory: sex differences in performance and monoaminergic activity. *Horm Behav* 43:48–59.
- Coburn-Litvak PS, Pothakos K, Tata DA, McCloskey DP, Anderson BJ. 2003. Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. *Neurobiol Learn Mem* 80:11–23.
- Conrad CD, Grote KA, Hobbs, RJ, Ferayorni A. 2003. Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobiol Learn Mem* 79:32–40.
- Czeh B, Welt T, Fischer AK, Erhardt A, Scmitt W, Muller MB, Toschi N, Fuchs E, Keck ME. 2002. Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. *Biol Psychiatry* 52: 1057–1065.
- Diamond DM, Bennett MC, Fleshner M, Rose GM. 1992.

- Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* 2:421–430.
- Drapeau E, Mayo W, Arousseau C, Le Moal M, Piazza P-V, Abrous DN. 2003. Spatial memory performance of aged rats in the water maze predicts levels of hippocampal neurogenesis. *Proc Natl Acad Sci USA* 100:14385–14390.
- Fuch E, Flugge G, Ohl F, Lucassen P, Vollmann-Honsdorf GK, Michaelis T. 2001. Psychosocial stress, glucocorticoids, and structural alterations in the tree shrew hippocampus. *Physiol Behav* 73:285–291.
- Gould E, Gross CG. 2002. Neurogenesis in adult mammals: some progress and problems. *J Neurosci* 22:619–623.
- Gould E, Tanapat P. 1999. Stress and hippocampal neurogenesis. *Biol Psychiatry* 46:1472–1479.
- Gould E, Tanapat P, Hastings NB, Shors TJ. 1999. Neurogenesis is adulthood: a possible role in learning. *Trends Cogn Sci* 3:186–192.
- Gundersen HJG, Jensen EB. 1987. The efficiency of systematic sampling in stereology and its predictions. *J Microsc* 147:229–263.
- Hoshooley JS, Sherry DF. 2004. Neuron production, neuron number and structure size are seasonably stable in the hippocampus of the food-storing black-capped chickadee (*Poecile atricapillus*). *Behav Neurosci* 118:345–355.
- Kempermann G. 2002. Why new neurons? Possible functions for adult hippocampal neurogenesis. *J Neurosci* 22:632–638.
- Kitaysky AS, Kitaikaia EV, Piatt JF, Wingfield JC. 2003. Benefits and costs of increased levels of corticosterone in seabird chicks. *Horm Behav* 43:140–149.
- Krebs JR, Clayton NS, Healy SD, Cristol DA, Patel SN, Jolliffe AR. 1996. The ecology of the avian brain: food-storing memory and the hippocampus. *Ibis* 138:34–46.
- Krebs JR, Sherry DF, Healy SD, Perry VH, Vaccarino AL. 1989. Hippocampal specialization of food-storing birds. *Proc Natl Acad Sci USA* 86:1388–1392.
- Lavenex P, Steele M, Jacobs LF. 2000. The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. *Eur J Neurosci* 12:643–648.
- Leverenz JB, Wilkinson CW, Wamble M, Corbin S, Grabber JE, Raskind, MA, Peskind ER. 1999. Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J Neurosci* 19:2356–2361.
- McEwen BS. 2000. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res* 886:172–189.
- McEwen BS. 2002. Sex, stress and the hippocampus: allostasis, allostatic load and the aging process. *Neurobiol Aging* 23:921–939.
- McEwen BS, Sapolsky RM. 1995. Stress and cognitive function. *Curr Opin Neurobiol* 5:205–216.
- Nottebohm F. 2002. Neuronal replacement in adult brain. *Brain Res Bull* 57:737–749.
- Ohl F, Michaelis T, Fujimori H, Frahm J, Rensing S, Fuchs E. 1999. Volumetric MRI measurements of the tree shrew hippocampus. *J Neurosci Methods* 88:189–193.
- Patel SN, Clayton NS, Krebs JR. 1997. Spatial learning induces neurogenesis in the avian brain. *Behav Brain Res* 89:115–128.
- Pravosudov VV. 2003. Long-term moderate elevation of corticosterone facilitates avian food-caching behavior and enhances spatial memory. *Proc R Soc Lond B* 270:2599–2604.
- Pravosudov VV, Clayton NS. 2001. Effects of demanding foraging conditions on cache retrieval efficiency in food-caching mountain chickadees (*Poecile gambeli*). *Proc R Soc Lond B* 268:363–368.
- Pravosudov VV, Clayton NS. 2002. A test of the adaptive specialization hypothesis: population differences in caching, memory, and the hippocampus in black-capped chickadees (*Poecile atricapilla*). *Behav Neurosci* 116:515–522.
- Pravosudov VV, Grubb TC Jr. 1997. Energy management in passerine birds during the non-breeding season: a review. *Curr Ornithol* 14:189–234.
- Pravosudov VV, Kitaysky AS, Wingfield JC, Clayton NS. 2001. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *Gen Comp Endocrinol* 123:324–331.
- Pravosudov VV, Lavenex P, Clayton NS. 2002. Changes in spatial memory mediated by experimental variation in food supply do not affect hippocampal anatomy in mountain chickadees (*Poecile gambeli*). *J Neurobiol* 51:142–148.
- Pravosudov VV, Mendoza SP, Clayton NS. 2003. The relationship between dominance, corticosterone, memory, and food caching in mountain chickadees (*Poecile gambeli*). *Horm Behav* 44:93–102.
- Pravosudov VV, Omanska A. 2004. Dominance-related changes in spatial memory are associated with changes in hippocampal cell proliferation rates in mountain chickadees. *J Neurobiol* (in press).
- Prickaerts J, Koopmans G, Blokland A, Scheepens A. 2004. Learning and adult neurogenesis: survival with or without proliferation? *Neurobiol Learn Mem* 81:1–11.
- Rakic P. 2002. Adult neurogenesis in mammals: an identity crisis. *J Neurosci* 22:614–618.
- Sapolsky RM. 1992. Neuroendocrinology of the stress response. In: Becker JB, Breedlove SM, Crews D, editors. *Behavioral endocrinology*. Cambridge, MA: MIT Press, p 287–324.
- Sapolsky RM. 1996. Why stress is bad for your brain. *Science* 273:749–750.
- Silverin B. 1998. Stress response in birds. *Poultry Avian Biol Rev* 9:153–168.
- Smulders TV, Sasson AD, DeVoogd TJ. 1995. Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J Neurobiol* 27:15–25.
- Smulders TV, Shiflett MW, Sperling AJ, DeVoogd TJ. 2000. Seasonal changes in neuron numbers in the hippocampal formation of a food-hoarding bird: the black-capped chickadee. *J Neurobiol* 44:414–422.

- Suosa N, Madeira MD, Paula-Barbosa MM. 1998. Effects of corticosterone treatment and rehabilitation on the hippocampal formation of neonatal and adult rats. An unbiased stereological study. *Brain Res* 794:199–210.
- Tanapat P, Hastings NB, Rydel TA, Galea LAM, Gould, E. 2001. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J Comp Neurol* 437:496–504.
- West MJ, Gundersen HJG. 1990. Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol* 296:1–22.
- West MJ, Ostergaard K, Andreassen OA, Finsen B. 1996. Estimation of the number of somatostatin neurons in the striatum: an in situ hybridization study using the optical fractionator method. *J Comp Neurol* 370:11–22.
- West MJ, Slomianka L, Gundersen HJG. 1991. Unbiased stereological estimation of the total number of neurons in the subdivision of the rat hippocampus using the optical fractionator. *Anat Rec* 231:482–497.
- Wingfield JC, Breuner C, Jacobs J. 1997. Corticosterone and behavioral responses to unpredictable events. In: Harvey S, Etches RJ, editors. *Perspectives in avian endocrinology*. Bristol: J. Endocrinol. Ltd., p 267–278.
- Wingfield JC, Maney DL, Breuner CW, Jacobs JD, Lynn S, Ramenofsky M, Richardson RD. 1998. Ecological bases of hormone-behavior interactions: the “emergency life history stage”. *Am Zool* 38:191–206.