



ACADEMIC
PRESS

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Hormones and Behavior 44 (2003) 93–102

Hormones
and Behavior

www.elsevier.com/locate/yhbeh

The relationship between dominance, corticosterone, memory, and food caching in mountain chickadees (*Poecile gambeli*)

Vladimir V. Pravosudov,^{a,*} Sally P. Mendoza,^a and Nicola S. Clayton^b

^a Department of Psychology, University of California at Davis, Davis, CA 95616-8519, USA

^b Department of Experimental Psychology, University of Cambridge, UK

Received 27 January 2003; revised 17 March 2003; accepted 18 March 2003

Abstract

It has been hypothesized that in avian social groups subordinate individuals should maintain more energy reserves than dominants, as an insurance against increased perceived risk of starvation. Subordinates might also have elevated baseline corticosterone levels because corticosterone is known to facilitate fattening in birds. Recent experiments showed that moderately elevated corticosterone levels resulting from unpredictable food supply are correlated with enhanced cache retrieval efficiency and more accurate performance on a spatial memory task. Given the correlation between corticosterone and memory, a further prediction is that subordinates might be more efficient at cache retrieval and show more accurate performance on spatial memory tasks. We tested these predictions in dominant–subordinate pairs of mountain chickadees (*Poecile gambeli*). Each pair was housed in the same cage but caching behavior was tested individually in an adjacent aviary to avoid the confounding effects of small spaces in which birds could unnaturally and directly influence each other's behavior. In sharp contrast to our hypothesis, we found that subordinate chickadees cached less food, showed less efficient cache retrieval, and performed significantly worse on the spatial memory task than dominants. Although the behavioral differences could have resulted from social stress of subordination, and dominant birds reached significantly higher levels of corticosterone during their response to acute stress compared to subordinates, there were no significant differences between dominants and subordinates in baseline levels or in the pattern of adrenocortical stress response. We find no evidence, therefore, to support the hypothesis that subordinate mountain chickadees maintain elevated baseline corticosterone levels whereas lower caching rates and inferior cache retrieval efficiency might contribute to reduced survival of subordinates commonly found in food-caching parids.

© 2003 Elsevier Inc. All rights reserved.

Keywords: Social dominance; Corticosterone; Mountain chickadee; Spatial memory; Food caching; Cache retrieval

Many animals that permanently reside in seasonally changing environments face energetic challenges during the winter as a result of limited and unpredictable food, low ambient temperature, and short days available for foraging. Wintering in such conditions places selection pressure on abilities to accumulate and maintain sufficient energy reserves. Most animals store energy reserves internally as body fat. In addition, some animals have evolved the ability to cache food externally, allowing them to accumulate and maintain potentially huge energy reserves as food caches

(Vander Wall, 1990; Pravosudov and Grubb, 1997). There are trade-offs associated with both of these strategies. Extra body fat appears to increase risk of predation related to decreased maneuverability and hence diminished ability to escape a predator attack, and increased needs to spend more time foraging in order to maintain heightened fat reserves (Lima, 1986; Witter and Cuthill, 1993; Pravosudov and Grubb, 1997). Food caches, on the other hand, need to be found and retrieved in order to be used, and most food-caching animals rely at least in part on spatial memory to find their previously made caches (e.g., Shettleworth, 1995; Krebs et al., 1996).

Many food-caching birds such as parids spend the winter in social groups characterized by a dominance hierarchy (Ekman, 1989). In such groups dominants enjoy priority

* Corresponding author. Department of Psychology, University of California at Davis, One Shields Avenue, Davis, CA 95616-8519, USA. Fax: +1-530-752-2087.

E-mail address: vpravosudov@ucdavis.edu (V.V. Pravosudov).

access to all food sources and, as a rule, dominants forage in more predator-safe areas compared to subordinates (Ekman, 1989). As a result, subordinate individuals should experience greater uncertainty in foraging success and therefore their perceived risk of starvation should be higher than that of dominants (Ekman and Lilliendahl, 1993). Consequently, subordinates should respond to the higher risk of starvation by increasing their fat reserves and food-caching rate (Pravosudov and Grubb, 1997; Brodin et al., 2001). The results with food-caching parids so far proved to be equivocal, however, with some studies showing that subordinates carry more fat reserves than dominants (Ekman and Lilliendahl, 1993), some finding no differences between subordinates and dominants (Pravosudov and Lucas, 2000), and others finding that dominants actually carry more fat reserves than subordinates (Koivula et al., 1995; Verhulst and Hogstad, 1996; Pravosudov and Lucas, 2000).

Similarly, some studies have shown more caching by dominants (Pravosudov, 1985; Hitchcock and Sherry, 1995; Lahti et al., 1998) while other studies showed no difference in caching rates (Pravosudov and Lucas, 2000) or that subordinates cache more than dominants (Lahti and Rytönen, 1996). One problem with some of the previous studies of captive birds is that the dominant and subordinate individuals were always tested together in a limited space where birds could not hide from each other. This is quite different from the natural situation, in which subordinates try to conceal their caching from the dominants (Lahti et al., 1998; Woodrey, 1991; Bugnyar and Kotrschal, 2002). It seems plausible that, like body fat (Witter and Swaddle, 1995), caching rates of dominants and subordinates might vary depending on environmental conditions. However, to fully understand the impact of food caching on energy budget of an individual food cacher we need to know whether the caches can be successfully retrieved. It is therefore critical to establish whether or not there are any differences between dominants and subordinates in cache retrieval efficiency, and in spatial memory accuracy.

It is well known that birds in energetically demanding conditions have elevated levels of corticosterone (Wingfield et al., 1997; Silverin, 1998). In particular, birds living in suboptimal foraging habitats (Marra and Holberton, 1998) and birds with food restrictions (Kitaysky et al., 1999; Reneerkens et al., 2002) have been reported to maintain elevated baseline levels of corticosterone. Pravosudov et al. (2001) also showed that limited and unpredictable food supply results in significantly elevated baseline corticosterone levels in mountain chickadees (*Poecile gambeli*) even after 2 months of experimental treatment, and that elevated levels of corticosterone triggered by unpredictable foraging conditions correlated with improved cache retrieval efficiency and with increased accuracy on a spatial but not nonspatial memory task (Pravosudov and Clayton, 2001; Pravosudov et al., 2001). In view of these findings we also predict that subordinates will maintain higher baseline corticosterone levels, retrieve their caches more efficiently, and

perform more accurately on a spatial but not nonspatial memory task compared to their dominant group mates. Some species have also been found to show stronger response to stress when living in harsher environmental conditions (Holberton and Able, 2002), so we may also predict that subordinates should have a stronger response to acute stress if they are challenged nutritionally. Overall, elevating corticosterone levels as a result of low social status could be an adaptation allowing low-ranking birds to manage their food caches more efficiently to offset the cost of subordination. Because elevated corticosterone has been linked with increased fattening in birds (Wingfield et al., 1997), higher levels of corticosterone in subordinate birds could explain their predicted higher fat reserves.

In this study, we specifically considered how dominance status affects food-caching rate, cache retrieval efficiency, spatial memory, and adrenocortical stress response in food-caching mountain chickadees. Although our study was carried out in captivity, we tried to avoid the pitfalls of the previous captive studies by testing each bird individually in isolation. We tested the following predictions: (1) subordinate birds should cache more than dominants; (2) subordinate birds should have more efficient cache retrieval, and more accurate performance on a spatial memory task than dominants; and (3) subordinate birds should have elevated baseline corticosterone levels and a stronger adrenocortical response to acute stress compared to dominants.

Methods

Twenty-four mountain chickadees were caught around Sage Hen, Tahoe National Forest, California, between November 14 and 18, 2001, using mist nets near the feeders. All birds were transported to the laboratory at the University of California in Davis and placed individually in wire-mesh cages (60 × 42 × 60 cm). All birds were maintained on a 8:16 h light:dark cycle at a constant 20°C temperature. Birds were fed with a mixture of pine nuts, shelled sunflower seeds, crushed peanuts, and mealworms, and given water with vitamins ad libitum.

After 21 days in captivity, we collected one capillary tube of blood from the brachial vein of each bird. We used plasma for radioimmunoassay analyses of corticosterone (see description below) and red blood cells to determine sex. We used Zoogen, Inc. (Davis, CA), for DNA sexing analyses. After we determined the sex of all individuals, we formed male–male and female–female pairs matched by wing length and placed birds from each pair individually in adjacent cages. These cages were separated by a metal partition which could be removed making one 120 × 42 × 60-cm cage in which two birds could freely interact. All cages were individually connected to a large experimental room (see description below). From December 30 to January 3, we allowed birds from the matched pairs to interact for the first time by allowing each pair into the experimental

room. We observed the birds through a one-way glass to determine dominance rank. We determined dominance rank by recording either aggressive interactions (one bird attacked the other whereas the other offered no resistance) or passive displacements (one bird always gives way to the other bird; Pravosudov and Lucas, 2000). After establishing the dominance status within each pair, birds were allowed to interact within their cages by removing the separating partition. As even the two cages combined provided somewhat limited space, we wanted to avoid excessive aggression, which could arise when birds start sharing the cage. Thus, at the beginning, we allowed birds to interact only for 2 h per day (25% of active time), gradually increasing this time to 6.5 h per day during 19 days (from January 4 to January 21, 2002). At the end, all birds still spent 1.5 h per day in isolation, which would allow them to feed without disturbance.

After 19 days of within-pair interactions when food was provided *ad libitum*, we started testing birds' memory performance individually in a cache recovery task and subsequently in two versions of a one-trial associative learning task. During the entire testing period, we maintained 6.5 h per day of interactions within all pairs. Thus, all birds interacted with their group mates every day but each bird was tested individually in order to ensure that, as in most natural conditions, caching decisions are not affected by the immediate presence of a group mate and that individual birds have a chance to cache food without being directly observed (Lahti et al., 1998). Prior to the behavioral tests, each bird was familiarized with the experimental room for 2 h each during two separate sessions. During these sessions food was provided *ad libitum* and birds were allowed to freely eat and cache. We also placed seeds in cache sites so that birds learned to search for food in these sites.

During the experiment, we made food more unpredictable for both birds by removing food for 2 h at a randomly determined time each day. There also was only one feeder available when two birds were in the same cage.

Experimental room

All birds were tested individually in the room (325 × 218 × 312 cm) and observed through a one-way Plexiglas window. The room contained 70 evenly spaced caching sites. There were two trees with 20 caching holes in each tree and 30 caching blocks which were hung from a wire mesh screen attached to the two opposite walls of the room (15 blocks on each wall). Each caching block consisted of a wooden rectangle (8 × 14 × 3.5 cm). In the center of the block there was a hole. Holes both in the trees and in wooden blocks were 0.5 cm in diameter and 0.5 cm deep. Each hole could be covered by a knot at the end of a short string attached above the hole to prevent the birds from seeing the content of the hole (Clayton, 1994). A wooden perch was located below each hole. The birds readily cached food in all caching sites and inspected them for food.

The experimenter manipulated the lights and opened the flaps connecting the home cages with the room so that the birds could fly in and out of the experimental room by flying from the dark to the light. Thus at the start of a trial, the lights were turned off in the home cage and turned on in the experimental room; at the end of the trial, the lights were reversed and the bird would fly into the home cage. By using this procedure, we avoided stress induced by handling birds.

Cache recovery task

The cache recovery task consisted of two phases separated by a retention interval of 5 h. Each bird was deprived of food 2 h before night roost and 1 h after waking up prior to Phase 1, in which birds were allowed to eat and to cache food individually in the experimental room with 70 available caching sites for 20 min. During Phase 1, an unlimited amount of pine nuts was provided in a bowl. After Phase 1, birds were returned to their home cages where they had *ad libitum* food (pine nuts, crushed peanuts, and sunflower seeds) and then deprived of food 2 h prior to Phase 2. During Phase 2, birds were allowed in the experimental room for 10 min and the only food available to them was that which they had cached during Phase 1. During Phase 2, all 70 caching holes were covered with a string knot so that the contents of caching sites were not visible. In order to retrieve a cache or to inspect the hole, a bird had to pull the string away from the hole. Prior to Phase 2, we placed all caches back in their original caching sites.

During the storage phase, we recorded the number of nuts cached and where they were located. During the recovery phase, we recorded the number of caches each bird recovered and the number of looks to find each nut. A bird was recorded as having made a look when it pulled at the string covering one of the potential cache sites. All cache recovery tests were performed from January 22 to February 15, 2002.

One-trial associative learning task

After the cache recovery trials were completed, we also tested each bird individually in a one-trial associative learning task. In this task, birds were trained to search in Phase 2 for food in sites in which they have found food previously during Phase 1 of a trial (Clayton and Krebs, 1994; Clayton, 1995; Pravosudov and Clayton, 2001, 2002). One problem with the cache recovery task is that if birds differed in the number of caches they make it would be more difficult to directly compare the birds' accuracy of cache recovery. In the one-trial associative learning task all birds have exactly the same experience because the experimenter effectively hides the caches and therefore controls for the number and location of items that need to be remembered.

We used two versions of the one-trial associative learning task. In both versions only one site was baited with food.

In version 1 (February 16–22, 2002), birds had 70 sites available to them exactly as in the cache recovery task including the same number of sites in trees and wooden blocks. The birds had to rely on memory in order to accurately return to the site that contained food. To examine the effect of motivation on performance, we also tested birds in a second version (February 23–25, 2000). We used 70 sites as in version 1, but this time the baited site was color-marked with a unique color (red and green) pattern so the birds did not have to rely on spatial memory to remember which feeder contained the hidden food in Phase 2. If birds differ in their motivation to search for food then they should differ in performance on both versions of the task. With the color-marked feeding site, birds would not have to search for food and instead they should search just for the marked site. If the two treatment groups differ in memory but not motivation then they should differ in performance in version 1 but not 2. To avoid a treatment order effect we performed another round of version 1 one-trial associative learning task after performing the version 2. If birds differed in memory but not in motivation, there should be significant difference between the treatment groups in both rounds of version 1 task but there should not be a difference in version 2.

Each trial consisted of two phases separated by a retention interval of 60 min. In Phase 1, each bird had to locate a visible piece of pine nut (which all birds did immediately without looking anywhere else). Each bird was allowed to eat a small part of the nut for 30 s, after which the lights in the experimental room were turned off and the bird returned to its home cage. All birds were deprived of food 2 h before night roost and 1 h after waking up and prior to Phase 1. Birds received no food between Phase 1 and Phase 2. In Phase 2, all caching sites were covered with a knot at the end of the string. If the bird remembered precisely where it had encountered the food, it should go directly to the site from which it had partially consumed the pine nut in Phase 1 (Clayton and Krebs, 1994). The site containing the nut in Phase 1 also contained pieces of pine nut covered by a string knot so the birds were rewarded for successful search. We recorded the number of sites inspected during Phase 2 for each bird.

Body mass

We weighted all birds in the morning (within 1 h after waking up) prior to forming social groups and at the end of the experiment in the morning (1 h after waking up) and in the evening (within 2 h before night roost). For each weighing, the birds were caught by hand and their body mass was measured on a Sartorius electronic balance with a precision of 0.01 g. As weighing required the birds to be handled, we avoided multiple mass measurements limiting our mass data to only three time points described above. As all pairs were

matched by wing length, we assumed that all changes in body mass within pairs reflected changes in relative fat reserves (Pravosudov and Grubb, 1997).

Corticosterone analyses

After all birds were maintained in captivity for 21 days and prior to forming social groups, we sampled baseline levels of corticosterone by collecting blood samples (approximately one capillary tube from each bird) from a brachial vein within 3 min of entering the cage, one bird at a time. All blood samples were collected from 10:00 to 12:00 to avoid time of day effects. After the behavioral experiments (approximately 15 weeks since bringing the birds into captivity), we collected blood samples on March 4–6, 2002. This time, we collected four blood samples from a brachial vein of each bird. Each bird was captured inside its cage and removed into a different room. The first sample was collected within 3 min of entering the cage, the second sample at 5 min, the third sample at 20 min, and the fourth sample at 50 min after entering the cage. The total amount of blood collected during the stress series did not exceed 1.5% of bird body mass, which should not cause an additional physiological stress to birds. All chickadees were held in cloth bags between sample collections. We used samples collected within 3 min of entering the cage to determine a baseline level of corticosterone because it has been shown that corticosterone levels usually do not start to elevate until 3 min after capture (Kitaysky et al., 1999; Pravosudov et al., 2001, 2002; see Results). Birds from both groups were bled at the same time of day (on average around 1300 for both groups, which is in the middle of active light hours) to avoid time bias for either treatment group.

We collected blood in heparinized capillary tubes and then emptied them into 0.3-ml vials that were kept on ice. All samples were centrifuged within 2 h of blood collection and the collected plasma samples were frozen at -20°C until assay.

Plasma corticosterone levels were estimated using ^{125}I radioimmunoassay kit from ICN Biomedicals (Cat. No. 07-120102, ICN Biomedicals, Costa Mesa, CA). Samples were assayed in duplicate. The intra- and interassay variance were 2.6 and 3.0%, respectively. The ICN corticosterone radioimmunoassay kit has been successfully used to analyze concentration of corticosterone in small avian plasma samples (Roy and Woolf, 2001; Washburn et al., 2002). In all these cases the ICN kit provided very reliable results. Cross-reactivity of the kit with corticosterone was 100% whereas it was $< 1\%$ for other steroids. In our study, we have also found the kit to provide reliable results under serial dilutions (parallelism) and the obtained patterns of adrenocortical stress response for mountain chickadees in this study were similar to the results previously obtained using Wingfield's protocols (Pravosudov et al., 2001, 2002).

Statistical analyses

As all pairs were matched by sex and wing length, we used a repeated-measures ANOVA with treatment and sex as factors for all behavioral analyses. When we had more than one trial, we took a mean across all trials. All data on feeding and caching rates as well as on number of searched cache sites were log transformed (natural logarithm) to meet the normality assumptions.

We used an ANCOVA to compare baseline levels of corticosterone between dominants and subordinates and to assess any potential effects of sex and time of day. We used a repeated-measures ANOVA (PROC MIXED; SAS Institute, 1994) to compare the entire adrenocortical stress response including all four samples taken within 50 min of entering the cage. All assumptions of statistical tests were upheld and the significance level was set at 0.05. For the main nonsignificant results we performed a power analysis. For the power analyses, we used previous work on chickadees which detected a significant increase in corticosterone levels of approximately 75% as a result of manipulations in food supply (Pravosudov et al., 2001). Thus, we calculated the statistical power to detect a 75% difference in baseline corticosterone levels.

Results

Dominance relationship

In all pairs, one bird immediately established as a dominant by winning all observed aggressive interactions (> 15 interactions observed for each pair, Sign test, $P < 0.001$).

Body mass

At the beginning of the experiment, there were no significant differences in morning body mass between dominants and subordinates but males weighed significantly more than females ($F_{1,10} = 47.6$, $P < 0.001$). At the end of the experiment, when males and females were combined, there were no significant differences in either morning (within 1 h after waking up) or evening (within 2 h before night roosting) body mass between dominants and subordinates, and the interaction between dominance status and sex was also nonsignificant. However, a posthoc LCD test revealed that within males, dominants tended to be heavier in the morning than subordinates and the difference bordered on significance ($P = 0.058$).

Eating and caching rates

There were no significant differences in food eating rates during the 20-min caching trials either between dominants and subordinates ($F_{1,10} = 3.6$, $P = 0.09$) or between males

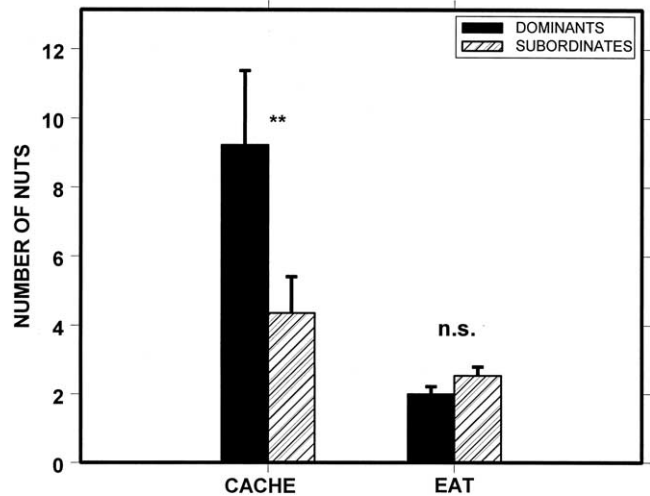


Fig. 1. Mean (\pm SEM) number of pine nuts eaten (completely or partially) and cached by dominant (solid bars, $n = 12$ for eating and $n = 11$ for caching rates) and subordinate (hatched bars, $n = 12$ for eating and $n = 11$ for caching rates) chickadees during the 20-min unlimited caching trials. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and females (Fig. 1). Dominants, however, cached significantly more food than subordinates ($F_{1,9} = 17.6$, $P < 0.01$; Fig. 1) and males also tended to cache more than females ($F_{1,9} = 10.9$, $P < 0.01$). The interaction between sex and dominance status was not statistically significant for both eating and caching rates. Analyses of independent contrasts showed that dominants cached significantly more food than subordinates within both male–male and female–female pairs ($P < 0.05$).

Cache retrieval

When we compared cache retrieval efficiency during the unlimited caching period, dominants made significantly fewer looks to find their caches ($F_{1,9} = 23.6$, $P < 0.001$) whereas there were no differences between males and females and the interaction between sex and dominance status was not statistically significant (Fig. 2). But because dominants cached more food, they could have retrieved proportionately more caches simply by chance as they had cached a greater number of nuts than the subordinates. To avoid this bias, after observing birds during the unlimited caching, we allowed all birds to cache only five nuts (limited caching), thus eliminating the difference in the number of caches between dominants and subordinates. Dominants again made significantly fewer looks to find their caches than subordinates ($F_{1,8} = 16.4$, $P < 0.01$; Fig. 2), and there was no significant difference between males and females and no significant interaction between sex and dominance status. As it is possible that some birds may have lost their motivation to search for food after consuming two or three caches, we also examined cache retrieval efficiency for the

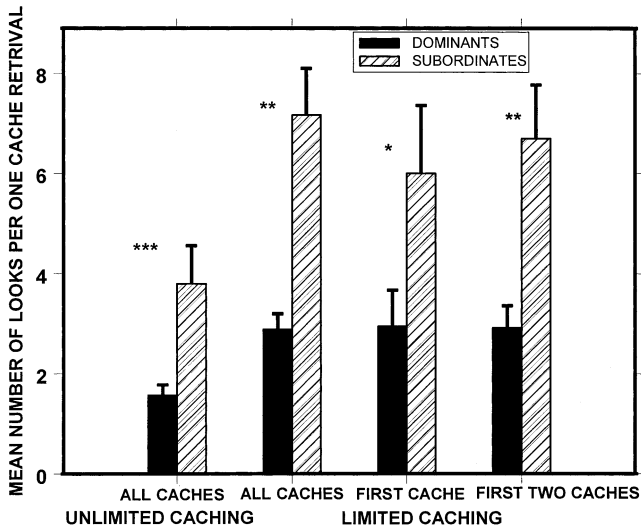


Fig. 2. Cache retrieval efficiency of dominant (solid bars) and subordinate (hatched bars) chickadees as expressed by a mean number of looks (\pm SEM) to find food caches in the unlimited ($n = 11$ for both dominants and subordinates) and limited ($n = 10$ for both dominants and subordinates) caching trials. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

first cache and for the first two caches birds found during cache retrieval sessions of limited caching trials. Supporting our previous finding, dominants made significantly fewer looks than subordinates in order to find either the first ($F_{1,8} = 7.6$, $P < 0.05$) or the first two caches ($F_{1,8} = 20.9$, $P < 0.01$; Fig. 2). Again, we found no significant differences between males and females and no significant interaction between sex and dominance status.

One-trial associative learning task

During version 1 of the one-trial associative learning task, dominants made significantly fewer looks than subordinates to find a pine nut that they had located previously ($F_{1,8} = 24.7$, $P < 0.001$; Fig. 3). There were no significant differences between males and females and no significant interaction between sex and dominance status. On version 2 of the one-trial associative learning task, when a site containing the nut was uniquely marked with green and red colors, there were no significant differences in memory performance between either dominants and subordinates ($F_{1,6} = 0.1$, $P = 0.82$) or between males and females as well as no significant interaction between sex and dominance status (Fig. 3). To ensure that this result did not occur simply because of a treatment order effect, we followed the color version with a repeat of version 1 of the one-trial associative learning task. In both trials of version 1, dominants showed significantly better performance than subordinates. Because birds' performance during the second trial of the version 1 was not significantly different from the first trial the results from both trials were combined.

Corticosterone

Corticosterone levels did not increase significantly in response to handling within 0–3 min interval after capture either during the first or last sampling; thus samples collected within 3 min of entering the cage were assumed to represent baseline values.

After 3 weeks in captivity and prior to forming social groups all birds had fairly high baseline levels of corticosterone (10.70 ± 1.67 ng/mL) which were significantly reduced by the end of experiment (3.52 ± 0.50 ng/mL; $F_{1,11} = 12.81$, $P < 0.01$). Neither dominance status nor sex had any significant influence on such a reduction.

There were no significant differences in baseline corticosterone levels between dominant and subordinate chickadees ($F_{1,15} = 0.18$, $P = 0.68$; Fig. 4) and this result appears to be fairly robust (Statistical Power = 0.81). There were no significant daily variations in baseline corticosterone for both groups of birds and there were no statistically significant differences between males and females. An interaction between dominance status and sex was also statistically significant. Body mass was not statistically related to baseline levels of corticosterone.

Dominant and subordinate chickadees did not differ significantly in the entire adrenocortical response to acute stress ($F_{1,20} = 0.8$, $P = 0.37$; Fig. 4). Both dominants and subordinates increased their levels of corticosterone significantly in response to the standardized acute stress ($F_{3,47} = 35.1$, $P < 0.001$; Fig. 4) but analyses of independent contrasts revealed that the increase was significant only up to 20 min after inducing acute stress (all P 's < 0.01), whereas there were no significant differences in corticosterone levels between 20 and 50 min since entering the cage (Fig. 4). An

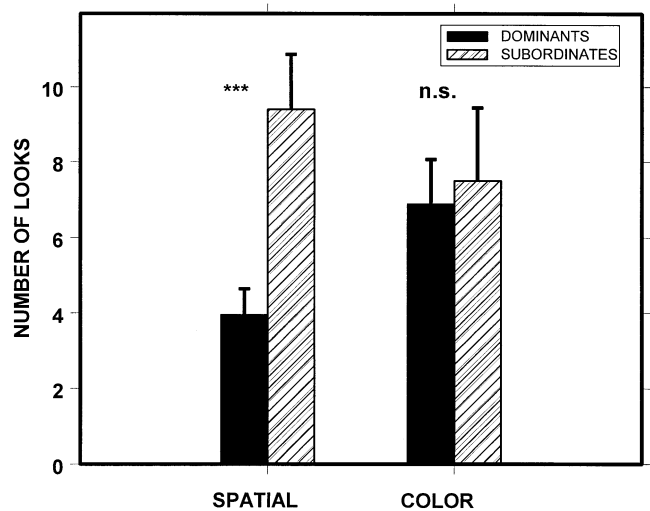


Fig. 3. Mean number of looks (\pm SEM) to find a food site for dominant (solid bars) and subordinate (hatched bars) chickadees in spatial ($n = 10$ for both dominants and subordinates) and nonspatial ($n = 8$ for both dominants and subordinates) versions of a one-trial associative learning task. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

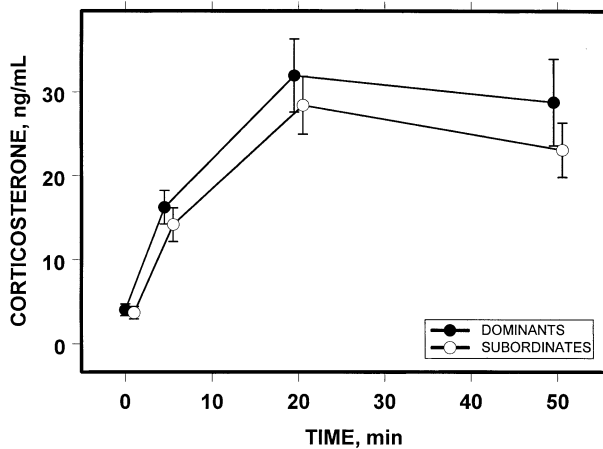


Fig. 4. Adrenocortical response to a standardized acute stress in dominant (solid circles, $n = 12$) and subordinate (open circles, $n = 12$) chickadees. Means \pm SEM.

interaction between dominance status and time since inducing stress was not statistically significant, suggesting that both dominants and subordinates had similar adrenocortical responses to acute stress. However, while some birds reached the highest levels of corticosterone already at 20 min, other birds reached highest levels only at 50 min after inducing acute stress. Thus, averaging all birds at each sampling time may not always represent the best way to compare the magnitude of the stress response and it is informative to compare the highest levels of corticosterone reached by each bird regardless of whether these levels were achieved by 20 or 50 min after inducing the stress. In our experiment dominants had significantly higher maximum reached levels of corticosterone during the entire stress response than subordinates (dominants— 36.73 ± 4.11 ng/mL; subordinates— 27.58 ± 1.65 ng/mL; $F_{1,18} = 4.51$, $P = 0.04$) whereas there were no significant differences between males and females and an interaction between sex and dominance status was also statistically insignificant. There were no significant differences between the sexes in their adrenocortical response to acute stress and interactions between dominance status and sex, time dominance status, and time since inducing stress were also not significant. Body mass also had no significant effect on maximum reached levels of corticosterone.

Discussion

In sharp contrast to all our predictions, this study demonstrated that subordinate mountain chickadees cached significantly less food than dominants and were less efficient at cache recovery than dominants. Subordinate chickadees also performed significantly less accurately than dominants on a spatial version of the one-trial associative learning task. By contrast, there were no significant differences between

dominants and subordinates when tested on a nonspatial version of the task in which a site containing food was color-marked. Dominants and subordinates also showed no differences in the baseline levels of corticosterone, but dominant birds reached significantly higher maximum levels of corticosterone during the stress response protocol.

A critical issue here is whether we can be certain that subordinate birds perceived their low social status and higher uncertainty of foraging success. In our experiment each pair was housed in a relatively small space (i.e., home cage) where they had to interact. Food was provided in only one feeder and access to food was unpredictably restricted in time to both the dominant and subordinate bird. Indeed, when we measured the dominance relationships, one member of each pair immediately become dominant by winning all aggressive interactions. Furthermore, dominants and subordinates demonstrated clear differences in caching rate and cache retrieval efficiency. All of the above suggests that subordinates perceived their low-ranking status.

Social dominance and food caching rate

The literature on the effect of dominance on caching rates provides mixed results (Pravosudov and Grubb, 1997; Brodin et al., 2001). As noted in the introduction, one possible reason for the differences between studies is that caching rates of dominants and subordinates might vary according to environmental conditions. Recently, Brodin et al. (2001) constructed a theoretical model, which predicted that in parids, subordinates should cache more food than dominants, but the entire model rests on a critical assumption that dominants can pilfer subordinates' caches but not vice versa. However, there is no empirical evidence supporting this assumption. Although parid group members separate their caching niches (Pravosudov, 1986; Lens et al., 1994), this separation is not absolute and there is a fair amount of overlap. Our study demonstrated that subordinate mountain chickadees cache significantly less than their dominant group mates even when the dominant birds could not have directly affected their caching behavior.

Memory or motivational differences?

There are a number of reasons that might explain the differences between dominant and subordinate individuals in cache retrieval efficiency and in performance on the one-trial associative memory tasks that we used. Dominant birds might possess a more accurate memory of the locations of their caches or they might be more motivated to search for food. Enhanced performance on memory tasks could also arise if dominant birds paid more attention to the cache sites during caching and, therefore, encoded the information more accurately, or because they were more accurate at recalling the sites in which they cached food. Our results do not distinguish between differences in attention at encoding and differences in memory recall.

Although dominants cached more food, there were no significant differences between dominants and subordinates in the amount of food they ate, suggesting that they had similar hunger levels. All birds were sufficiently motivated to search for hidden food, and there was no difference between dominants and subordinates when tested for motivation levels in the nonspatial version of the one-trial associative learning task in which a baited site was uniquely color marked. Although this result suggests that dominants and subordinates were equally motivated to search for hidden food, it remains possible that the test was not sensitive enough to detect possible differences in motivation.

Corticosterone and cache retrieval efficiency

Our prediction that subordinate birds would have higher baseline levels of corticosterone and better spatial memory was based on our previously found correlations between corticosterone and memory (Pravosudov and Clayton, 2001; Pravosudov et al., 2001). Because unpredictable food supply was shown to result in elevated baseline levels of corticosterone (Pravosudov et al., 2001), we expected subordinates, which should perceive their foraging opportunities less predictable, to have elevated baseline corticosterone compared to dominant birds. Similarly, we expected subordinates to show more efficient cache retrieval than dominants based on the result that mountain chickadees maintained on unpredictable food, which caused elevation in baseline corticosterone, showed more efficient cache retrieval and better spatial memory performance (Pravosudov and Clayton, 2001). Our study demonstrated that in spite of no differences in baseline corticosterone levels, subordinate chickadees were less efficient at cache retrieval efficiency and showed less accurate performance on a spatial memory task than their dominant group mates. This result suggests that dominance-induced differences in spatial memory in food-caching birds are not necessarily related to changes in glucocorticoid hormones. It is possible that corticosterone levels in subordinate birds were significantly elevated when social groups were initially formed and then eventually reduced at the end of the experiment. Several studies showed that birds could reduce corticosterone levels after a prolonged exposure to nutritional stress (Carsia and McIlroy, 1998; Kitaysky et al., 2001). Subordinates could have also reduced their corticosterone levels as the dominance relationships stabilized (Mendoza et al., 2000; Creel, 2001). It is possible that elevation of corticosterone during the initial phases of subordination could have produced a long lasting effect on the hippocampus and spatial memory performance (McEwen and Sapolsky, 1995). On the other hand, our previous study demonstrated that even after 2 months of unpredictable food mountain chickadees maintained significantly elevated baseline corticosterone levels (Pravosudov et al., 2001), which suggests that if social subordination was based on a perception of foraging conditions, we should have detected elevated corticosterone

even after a prolonged period of time. An alternative explanation is that dominant birds are a priori of higher quality than subordinates.

Dominance status and corticosterone

Despite the assumption that subordinates should experience higher stress as a result of higher uncertainty in foraging success and despite the fact that in our experiment food access was restricted for both dominants and subordinates, we failed to find a difference in baseline corticosterone levels between these two groups with a robust statistical power (>80%). Available data on the relationship between glucocorticoid hormones and dominance status are also ambiguous (Creel, 2001). Some studies showed that subordinates have higher baseline levels of glucocorticoid hormones (Rohwer and Wingfield, 1981; Schwabl et al., 1988; Belthoff and Dufty, 1994), whereas other studies failed to find a difference between dominants and subordinates (Hegner and Wingfield, 1987; Holberton et al., 1989; Stavisky et al., 2001; Lynch et al., 2002) or found that dominants have higher baseline glucocorticoid levels (Creel, 2001). Mendoza et al. (2000) argued that stable dominance relationships in nonhuman primates, for example, are not stressful to subordinate members of a group but rather instability of social relationships may result in chronic stress and elevated glucocorticoid hormones. Thus, it is not clear in which cases we can predict subordinates in stable social groups to have elevated levels of corticosterone as this experiment demonstrated that the simple presence of a dominance relationship does not ensure such a result. Interestingly, Rohwer and Wingfield (1981) found that negative correlation between dominance status and corticosterone levels in Harris' sparrows (*Zonotrichia querula*) was almost reversed after heavy snowfall, suggesting that the relationship between dominance status and corticosterone may depend on environmental and social conditions (Creel, 2001).

Dominance and magnitude of adrenocortical stress response

We found that maximum corticosterone levels reached during the stress response protocol were significantly higher for dominant chickadees. Higher response of the HPA axis to stress is usually associated with more unpredictable or potentially harsher environmental conditions (e.g., Holberton and Able, 2002). If the environment of subordinates were less predictable and harsher, we would expect subordinates to have a stronger adrenocortical stress response. Supporting this view, Schwabl et al. (1988) found that low ranking white-throated sparrows (*Z. albicollis*) had a more rapid adrenocortical stress response than higher-ranking birds. Our results contrast with these findings. It is possible that dominants can benefit from more sensitive HPA axis and they can sense and respond quicker to deterioration in

environmental conditions, especially if these conditions were already harsh. This hypothesis contradicts our previous explanation of higher adrenocortical stress response in female chickadees compared to males, which we assumed was due to their lower social rank (Pravosudov et al., 2001, 2002). However, the magnitude of adrenocortical response to stress in birds of different social rank may also vary depending on prevailing environmental conditions. In either case, we clearly need more data to understand why dominants might have heightened adrenocortical response to acute stress.

Acknowledgments

V.V.P. was supported by a National Research Service Award Postdoctoral Fellowship from the National Institute on Drug Abuse and by a career grant (K01 MH 65984) from the National Institute of Mental Health at the National Institutes of Health. N.S.C. was supported by grants from the National Institutes of Health NS 35465-05 and MH2602, and BBSRC Grant S16565. Birds were collected under California State and Federal Scientific Collecting Permit 801080-01 to V.V.P. and a permit from Tahoe National Forest (00-MU-11051760-009). All experiments were performed in accordance with the University of California at Davis animal care protocol 8812.

References

- Belthoff, J.R., Dufty Jr., A.M., 1994. Plumage variation, plasma steroids and social dominance in male house finches. *Condor* 96, 614–625.
- Brodin, A., Lundborg, K., Clark, C.W., 2001. The effect of dominance on food hoarding: a game theoretical model. *Am. Nat.* 157, 66–75.
- Bugnyar, T., Kotschal, K., 2002. Observational learning and the raiding of food caches in ravens, *Corvus corax*: is it 'tactical' deception? *Anim. Behav.* 64, 185–195.
- Carsia, R.V., McLroy, P.J., 1998. Dietary protein restriction stress in the domestic turkey (*Meleagris gallopavo*) induces hypofunction and remodeling of adrenal steroidogenic tissue. *Gen. Comp. Endocr.* 109, 140–153.
- Clayton, N.S., 1994. The role of age and experience in the role of development of food storing and retrieval in marsh tits. *Parus palustris*. *Anim. Behav.* 47, 1435–1444.
- Clayton, N.S., 1995. Development of memory and the hippocampus: comparison of food-storing and nonstoring birds on a one-trial associative memory task. *J. Neurosci.* 15, 1796–1807.
- Clayton, N.S., Krebs, J.R., 1994. One-trial associative memory: comparing of food-storing and nonstoring species of birds. *Anim. Learn. Behav.* 22, 366–372.
- Creel, S., 2001. Social dominance and stress hormones. *Trends Ecol. Evol.* 16, 491–497.
- Ekman, J., 1989. Ecology of non-breeding social systems of *Parus*. *Wilson Bull.* 101, 263–288.
- Ekman, J.B., Lilliendahl, K., 1993. Using priority to food access: fattening strategies in dominance-structured willow tit (*Parus montanus*) flocks. *Behav. Ecol.* 4, 232–238.
- Hegner, R.E., Wingfield, J.C., 1987. Social status and circulating levels of hormones in flocks of house sparrows. *Passer domesticus*. *Ethology* 76, 1–14.
- Hitchcock, C., Sherry, D.F., 1995. Cache pilfering and its prevention in pairs of black-capped chickadees. *J. Avian Biol.* 26, 187–192.
- Holberton, R.L., Able, K.P., 2002. Differential migration and an endocrine response to stress in wintering dark-eyed juncos (*Junco hyemalis*). *Proc. R. Soc. London B* 268, 1889–1896.
- Holberton, R.L., Able, K.P., Wingfield, J.C., 1989. Status signaling in dark-eyed juncos, *Junco hyemalis*: plumage manipulations and hormonal correlates of dominance. *Anim. Behav.* 37, 681–689.
- Kitaysky, A.S., Kitaiskaia, E.V., Wingfield, J.C., Piatt, J.F., 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. *J. Comp. Physiol. B* 171, 701–709.
- Kitaysky, A.S., Piatt, J.F., Wingfield, J.C., Romano, M., 1999. The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restrictions. *J. Comp. Physiol. B* 169, 303–310.
- Koivula, K., Orell, M., Rytönen, S., Lahti, K., 1995. Fatness, sex and dominance; seasonal and daily body mass changes in willow tits. *J. Avian Biol.* 26, 209–216.
- Krebs, J.R., Clayton, N.S., Healy, S.D., Cristol, D.A., Patel, S.N., Jolliffe, A.R., 1996. The ecology of the avian brain: food-storing memory and hippocampus. *Ibis* 138, 34–46.
- Lahti, K., Koivula, K., Rytönen, S., Mustonen, T., Welling, P., Pravosudov, V.V., Orell, M., 1998. Social influences on food caching in willow tits: a field experiment. *Behav. Ecol.* 9, 122–129.
- Lahti, K., Rytönen, S., 1996. Presence of conspecifics, time of day and age affect willow tit hoarding. *Anim. Behav.* 52, 631–636.
- Lens, L., Adriansen, F., Dhondt, A.A., 1994. Age-related hoarding strategies in the crested tit *Parus cristatus*: should the cost of subordination be reassessed? *J. Anim. Ecol.* 63, 749–755.
- Lima, S.L., 1986. Predation risk and unpredictable foraging conditions: determinants of body mass in birds. *Ecology* 67, 377–385.
- Lynch, J.W., Ziegler, T.E., Strier, K.B., 2002. Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys. *Cebus apella nigratus*. *Horm. Behav.* 41, 275–287.
- McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. *Curr. Opin. Neurobiol.* 5, 205–216.
- Marra, P.P., Holberton, R.L., 1998. Corticosterone levels as indicators of habitat quality: effects of habitat segregation in a migratory bird during the non-breeding season. *Oecologia* 116, 284–292.
- Mendoza, S.P., Capitanio, J.P., Mason, W.A., 2000. Chronic social stress: studies in non-human primates, in: Moberg, G.P., Mench, J.A. (Eds.), *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare*. CABI Publishing, New York, pp. 227–247.
- Pravosudov, V.V., 1985. Search for and storage of food by *Parus cinctus lapponicus* and *P. montanus borealis*. *Zool. Zh.* 64, 1036–1043.
- Pravosudov, V.V., 1986. Individual differences in foraging and storing behavior in Siberian tit *Parus cinctus* Bodd. and willow tit *Parus montanus* Bold. *Soviet J. Ecol.* 4, 60–64.
- Pravosudov, V.V., Clayton, N.S., 2001. Effects of demanding foraging conditions on cache retrieval accuracy in food-caching mountain chickadees (*Poecile gambeli*). *Proc. R. Soc. London B* 268, 363–368.
- Pravosudov, V.V., Clayton, N.S., 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, V.V., Grubb Jr., T.C., 1997. Energy management in passerine birds during the non-breeding season: a review. *Curr. Ornithol.* 14, 189–234.
- Pravosudov, V.V., Kitaysky, A.S., Wingfield, J.C., Clayton, N.S., 2001. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). *Gen. Comp. Endocrinol.* 123, 324–331.
- Pravosudov, V.V., Kitaysky, A.S., Saldanha, C.J., Wingfield, J.C., Clayton, N.C., 2002. The effect of photoperiod on adrenocortical stress response in mountain chickadees (*Poecile gambeli*). *Gen. Comp. Endocrinol.* 126, 242–248.

- Pravosudov, V.V., Lucas, J.R., 2000. The effect of social dominance on fattening and food-caching behavior in Carolina chickadees. *Poecile carolinensis*. *Anim. Behav.* 60, 483–493.
- Reneerkens, J., Piersma, T., Ramenofsky, M., 2002. An experimental test of the relationship between temporal variability of feeding opportunities and baseline levels of corticosterone in a shorebird. *J. Exp. Zool.* 293, 81–88.
- Rohwer, S., Wingfield, J.C., 1981. A field study of social dominance, plasma level of luteinizing hormone and steroid hormones in wintering Harris' sparrows. *Z. Tierpsychol.* 57, 173–183.
- Roy, C., Woolf, A., 2001. Effects of hunting and hunting-hour extension on mourning dove foraging and physiology. *J. Wildlife Management* 65, 808–815.
- Schwabl, H., Ramenofsky, M., Schwabl-Benzinger, I., Farnier, D.S., Wingfield, J.C., 1988. Social status, circulating levels of hormones, and competition for food in winter flocks of the white-throated sparrow. *Behaviour* 107, 107–121.
- Shettleworth, S.J., 1995. Memory in food-storing birds: from the field to the Skinner box, in: Alleva, E., Fasolo, A., Lipp, H.P., Nadel, L., Ricceri, L. (Eds.), *Behavioral Brain Research in Naturalistic and Semi-naturalistic Settings*. Kluwer, The Hague, pp. 158–179.
- Silverin, B., 1998. Stress response in birds. *Poultry Avian Biol. Rev.* 9, 153–168.
- Stavisky, R.C., Adams, M.R., Watson, S.L., Kaplan, J.R., 2001. Dominance, cortisol, and behavior in small groups of female cynomolgus monkeys (*Macaca fascicularis*). *Horm. Behav.* 39, 232–238.
- Vander Wall, S.B., 1990. *Food Hoarding in Animals*. University of Chicago Press, Chicago.
- Verhulst, S., Hogstad, O., 1996. Social dominance and energy reserves in flocks of willow tits. *J. Avian Biol.* 27, 203–208.
- Washburn, B.E., Morris, D.L., Millsbaugh, J., Faaborg, J., Schulz, J.H., 2002. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. *Condor* 104, 558–563.
- Wingfield, J.C., Breuner, C., Jacobs, J., 1997. Corticosterone and behavioral responses to unpredictable events. in: Harvey, S., Etches, R.J. (Eds.), *Perspectives in Avian Endocrinology*. J. Endocrinol. Ltd., Bristol, pp. 267–278.
- Witter, M.S., Cuthill, I.C., 1993. The ecological costs of avian fat storage. *Philos. Trans. R. Soc. London B* 340, 73–90.
- Witter, M.S., Swaddle, J.P., 1995. Dominance, competition and energetic reserves in the European starling. *Sturnus vulgaris*. *Behav. Ecol.* 6, 343–348.
- Woodrey, M.S., 1991. Caching behavior in free-ranging white-breasted nuthatches: the effect of social dominance. *Ornis Scand.* 22, 160–166.