



Photoperiodic regulation of food storing and hippocampus volume in black-capped chickadees, *Poecile atricapillus*

SCOTT A. MACDOUGALL-SHACKLETON*, DAVID F. SHERRY†, ANDREW P. CLARK*, REBECCA PINKUS† & ALEXANDRA M. HERNANDEZ*

*Department of Psychology, University of Toronto at Mississauga

†Department of Psychology, University of Western Ontario

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In seasonal environments animals organize their behaviour around annual cycles of resource availability. Wild black-capped chickadees are most likely to hoard food in autumn. At this time of year chickadees are also reported to have a larger hippocampus, a brain area important for spatial memory. This study examined how photoperiodic condition affects these seasonal changes. Captive chickadees were exposed to one of three treatments. Photorefractory birds were held on long days (19:5 h light:dark) and had small gonads. Photosensitive birds were held on short days (LD 9:15 h) and also had small gonads. Photostimulated birds were switched from short to long days and quickly entered breeding condition with large gonads. Photosensitive birds (on short days) stored more seeds than photorefractory birds (on long days). Photostimulated birds stored seeds at a high rate when on short days, but reduced storing when transferred to long days. These results indicate that long days inhibit storing regardless of gonadal condition. There were no differences between groups in hippocampal volume, indicating that photoperiod can produce changes in food-storing behaviour without affecting hippocampal size.

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Most organisms live in environments that show seasonal changes in resource availability and as a result organize their behaviour in response to these cyclic resource changes. For example, most animals time breeding such that young are produced during the times of year when resources important for offspring growth are most abundant (Lack 1968). As well, many animals migrate when resources are scarce or enter states of relative inactivity (such as hibernation or estivation). Another adaptation to resource scarcity is to cache food resources when they are abundant for use later when they become scarce. Anecdotal data suggest that black-capped chickadees scatterhoard food primarily during the autumn and winter and retrieve these resources when food is scarce (Odum 1942). The annual cycle in several species of titmice appears to involve a seasonal change in food storing and retrieval (Odum 1942; Haftorn 1956; Ludescher 1980; Pravosudov 1985) with food storing peaking in October (Nakamura & Wako 1988). In this study we address the mechanisms by which black-capped chickadees time their annual change in food storing.

In black-capped chickadees seasonal changes have been reported in both food storing (Odum 1942; Shettleworth

et al. 1995) and in the hippocampus (Barnea & Nottebohm 1994; Smulders et al. 1995, 2000b), a brain area important for spatial memory and cache retrieval (e.g. Sherry & Vacarino 1989; Hampton & Shettleworth 1996). However, the environmental cues that drive these changes and the mechanisms by which such cues are transduced are relatively unexplored. Because it is so important for timing annual changes in reproductive physiology, and based on prior work (Shettleworth et al. 1995; Clayton & Cristol 1996), the annual change in photoperiod is a good candidate as a cue that regulates changes in food storing. A large body of research on how photoperiod regulates seasonal changes in reproduction has provided a theoretical framework that may prove useful, and is briefly reviewed here.

Photoperiod and Seasonality in Birds

Most of our understanding of how birds use photoperiod to organize annual changes in behaviour stems from work on seasonal reproduction. The factors regulating seasonal changes in reproduction can be divided into ultimate and proximate factors (Bronson 1989). Ultimate factors are those environmental variables that result in differential reproductive success, whereas proximate factors are the environmental cues that organisms actually

Correspondence and present address: S. A. MacDougall-Shackleton, Department of Psychology, University of Western Ontario, London, ON N6A 5C2, Canada (email: smacdou2@uwo.ca).

use to time reproduction. These may or may not be the same. For example, if food abundance determines reproductive success and animals directly respond to food to time reproduction, food is both an ultimate and proximate factor. Although food abundance is a very common ultimate factor, many species use other proximate environmental cues to predict food abundance and time reproduction. Similarly, although food abundance may be the ultimate factor causing food caching, cues other than food itself may regulate seasonal changes in the expression of the behaviour.

Almost all seasonally breeding songbirds use changes in photoperiod as the major initial predictive cue to time reproduction (Wingfield 1983). Other cues, such as food supply, temperature, nest site availability or social interactions, tend to modify seasonal changes in reproductive physiology and behaviour that are principally driven by changes in photoperiod (Wingfield 1983; Wingfield & Kenagy 1991). In the temperate zone seasonally breeding songbirds experience short days in late winter, but are in a state of responsiveness to long days and are said to be photosensitive. In spring, when days become long enough to illuminate the photo-inducible phase of the circadian cycle, songbirds show a surge in gonadotropic hormones, grow their gonads, increase circulating sex steroid levels and engage in reproductive behaviours (reviewed in Nicholls et al. 1988). However, later in summer while days are still long, seasonally breeding songbirds cease responding to long days, regress their gonads and initiate feather moult. At this point, the bird is said to be photorefractory, and in some species, birds must experience short days before they will cease being photorefractory and once again become photosensitive (Nicholls et al. 1988). Thus, seasonally breeding songbirds' reproductive physiological state can be dissociated from time of year by maintaining birds on short days and thus in a long-term photosensitive state, or by maintaining birds on long days and thus in a long-term photorefractory state.

The annual changes in sensitivity to long days that drive birds through states of photosensitivity, photostimulation and photorefractoriness coordinate annual changes in a wide array of behaviours and physiological processes including reproduction, singing, moult and seasonal migration (e.g. Emlen 1969; Wingfield & Kenagy 1991; Ball 1999). The purpose of this study was to examine how changes in photoperiod and photoperiodic state interact to regulate food-storing behaviour and hippocampus morphology in black-capped chickadees. Specifically, we compared food storing and hippocampus volume among three groups of birds: (1) long-day photorefractory birds, (2) short-day photosensitive birds, and (3) birds transferred from short to long days and thereby photostimulated. We did not examine any differences in spatial memory among these groups. These groups were chosen so that we could directly assess the relative contribution of photoperiodic state and daylength to food storing and hippocampus structure. It is possible that food storing decreases in spring under long day conditions because of the onset of reproductive activity. The photostimulated and photorefractory groups permit a

comparison of reproductive and nonreproductive birds both on long days. Chickadees store most in the autumn when they might still be photorefractory and when days are short. In this study we assessed the influence of photorefractoriness and short days independently by observing food storing and hippocampal size in photorefractory birds on long days and in photosensitive birds on short days.

Previous attempts to induce change in hippocampal size by manipulating photoperiod maintained birds on long days and then shortened the day length to simulate autumn photoperiod (Krebs et al. 1995). While this manipulation increased food storing it had no effect on hippocampal size, perhaps because natural levels of food storing and retrieval could not be attained in the laboratory (Krebs et al. 1995). In this experiment we increased day length to simulate spring conditions in the photostimulated group to determine whether this manipulation would produce a decrease in food storing, and potentially, a decrease in hippocampal size as well.

METHODS

Animals

We captured nine male and nine female black-capped chickadees in walk-in feeder traps in the vicinity of Mississauga, Ontario, Canada (43°36'N, 79°39'W) under Environment Canada scientific capture permit CA0065. Birds were initially housed in groups of two or three, then moved to individual cages prior to the experiment. Birds were sexed by laparotomy under anaesthesia (Isoflurane) shortly after capture. By the onset of the experiment (in February and March 2000) all birds were adults. Throughout the study, we fed birds ground sunflower seeds and Mazuri Small Bird Maintenance (Purina) ad libitum. Prior to behavioural tests, we occasionally supplemented the birds with fresh greens or carrots; we ceased these supplements during testing. Food and water were replenished daily. The animal care committee of the University of Toronto approved all animal care procedures.

Photoperiod Manipulation

Prior to 12 November 1999 the birds' light schedule mimicked the naturally declining photoperiod for Mississauga. We assigned the birds to three groups, balanced by sex. Due to a timer failure, several birds in one room experienced 24-h light cycle from 12–16 November 1999. Following this, and until the behavioural testing phase, we maintained six of these birds (3 males and 3 females captured between 16 October and 12 November) on an LD 19:5 h cycle. These six birds comprised the photorefractory group. We used 12 other birds (6 males and 6 females captured between 16 October and 7 December) that had not been exposed to long days for the photosensitive and photostimulated groups. These birds experienced an LD 8.75:15.25 h photoperiod from their time of capture until the behavioural testing phase. These photoperiod regimes were maintained until 6 February

2000, at which point behavioural testing began. By this time, birds in the photorefractory group had been on constant long days for 12 weeks, and birds in the other two groups had been on naturally declining short days for several months in the wild, followed by constant short days in the laboratory for 9–16 weeks.

Behavioural testing was carried out in two phases. Throughout phase 1 all birds were maintained on the same constant photoperiod they had experienced before testing. During phase 2, birds in the photostimulated group were transferred to an LD 19:5 h photoperiod and the other two groups continued with their prior photoperiod. Thus, the photorefractory birds experienced long days throughout the experiment, the photosensitive birds experienced short days throughout the experiment, and the photostimulated birds experienced short days followed by a switch to long days part way through the experiment.

Behavioural Measures

Every 4 days, we provided each bird an opportunity to store seeds. Storing took place in a flight cage (180 × 76 × 185 cm) in a separate room from the home cages. The flight cage contained six horizontal pieces of wood with 10 holes (width=0.7 cm; depth=1.3 cm) drilled in each. A small perch was mounted below each hole. During the storing task, we transferred each bird to the flight cage and provided it access to a dish of hulled sunflower seeds for 30 min. After this time, we recorded the number of stored seeds. Storing sessions began on 6 February 2000 and ended on 20 March 2000. Phase 1 of the experiment included seven storing sessions, and phase 2 of the experiment included four storing sessions. Following phase 2 all birds were killed and their brains extracted (see below). Thus, at the time of brain extraction the photorefractory birds had been on long days for about 18.5 weeks, the photosensitive birds had been on short days for over 22 weeks, and the photostimulated birds had been on long days for 16 days following more than 20 weeks of short days.

Histological Measures

On 21 March 2000 all birds were deeply anaesthetized with a lethal dose of sodium pentobarbital, and transcardially perfused with heparinized 0.1 M phosphate-buffered saline (pH 7.5) followed by buffered 4% paraformaldehyde (pH 8.5). We then extracted, cryoprotected (in 30% sucrose in buffer) and froze the brains on pulverized dry ice. Brains were stored at -80°C until processing.

Following perfusion we dissected out and measured the gonads of each bird. For males, we measured the length and width of the left testis, and computed testicular volume using the formula $V=4/3\pi a^2 b$ where a is half the width and b is half the length. For females we scored ovarian development using the following scale: 1, smooth, no visible follicular development; 2, slightly granular appearance; 3, small follicles apparent, but no

follicular hierarchy; 4, obvious follicles with evident hierarchy; 5, large yolky follicles. The individual measuring the gonads was kept blind to the treatment group of each bird.

We sectioned brains in the coronal plane at -17°C in a cryostat. Sections were 40 μm thick and we collected every second section throughout the telencephalon, starting 1.6 mm from the rostral-most tip of the telencephalon and continuing until the caudal-most part of the telencephalon. We mounted sections on to gelatin-coated microscope slides, stained the sections with thionin, and then protected the sections with coverslips mounted with Permout (Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.).

Our reanalysis of previous data indicate that the rostral-most portion of the brain not collected in this study represents a small portion of the telencephalon and that estimates of telencephalon size with or without this rostral tip are tightly correlated. For example, reanalysis of previously published data (Petersen & Sherry 1996) indicate that estimates of telencephalon size with or excluding the rostral tip are tightly and linearly related (Fisher's r to z transformation: $r=0.98$, $N=23$, $P<0.0001$). Thus, estimation of telencephalon size without this rostral-most tip should not influence the analyses using general linear models as reported below.

Brain Morphometry

The volume of the hippocampus, comprising the hippocampal formation and the area parahippocampalis (Sherry et al. 1989), and the volume of the telencephalon (excluding hippocampus) was determined for each bird by measuring the area of these regions on every second mounted section (i.e. at 160 μm intervals) and combining area estimates using the formula for a truncated cone to obtain volume of the structure. Areas of the hippocampus and telencephalon were digitized using a 1.25 × objective on a Zeiss Axiophot microscope, a SONY Hyper HAD digital colour video camera and Sigma Scan Pro software. The volume of the left and right hippocampus, and the left and right telencephalon, were determined separately then summed to obtain total volume for each structure. Volume estimates for the hippocampus were obtained from a mean of 39.5 sections per brain and for the telencephalon from a mean of 44.0 sections per brain. The observer was blind to the experimental treatment of each bird.

RESULTS

Photoperiod Manipulation

The gonadal state of the birds indicated that the photoperiod manipulations had the intended effects. That is, birds held on long days long-term (photorefractory group) had fully regressed gonads, birds held on short days long-term (photosensitive group) also had regressed gonads, and birds switched from short to long days (photostimulated group) had enlarged gonads in breeding condition (Fig. 1). Male testis volume varied significantly between groups (one-way ANOVA: $F_{2,6}=9.28$,

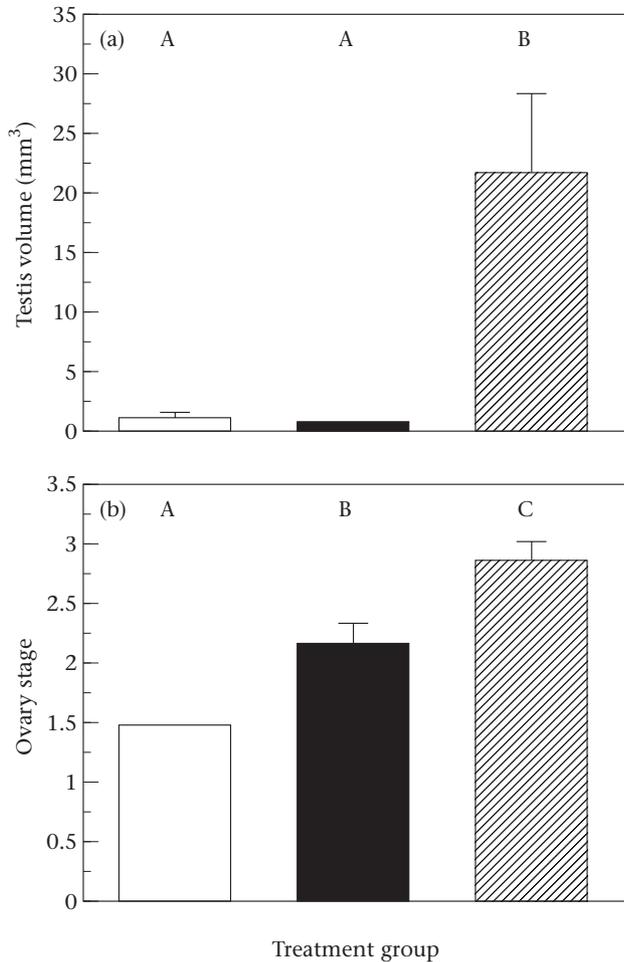


Figure 1. Gonad size (\bar{X} +SE) of male and female photorefractory (□), photosensitive (■) and photostimulated (▨) black-capped chickadees. In some cases error bars are too small to be visible. Bars marked with different letters differed significantly from each other.

$P=0.01$) and Scheffe's post hoc tests indicated that the photostimulated birds had larger testes than either photorefractory or photosensitive birds, which did not differ from each other. Ovary stage also varied significantly between groups ($F_{2,6}=24$, $P=0.001$), with photostimulated females having the most developed ovaries. Scheffe's post hoc tests showed that photosensitive females had significantly greater ovarian development than photorefractory females, but significantly less development than photostimulated females ($P<0.05$).

Food Storing

Birds on short days stored more seeds than birds on long days, regardless of gonadal condition (Fig. 2). To compare the rate of storing among groups between phase 1 and phase 2 of the experiment, we calculated for each bird the mean number of stored seeds over the last four sessions of phase 1 and the mean number of stored seeds over the first four sessions of phase 2. A two-way repeated measures ANOVA revealed a significant main effect of treatment group ($F_{2,15}=6.38$, $P=0.01$), a significant main

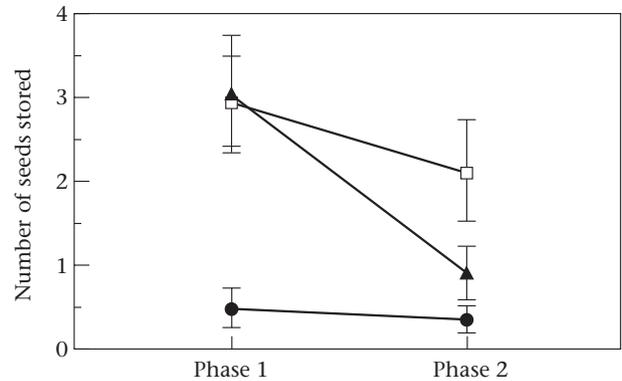


Figure 2. Mean (\pm SE) number of seeds stored per session for photosensitive (□), photostimulated (▲) and photorefractory (●) black-capped chickadees. A two-way ANOVA indicated a main effect of treatment group, a main effect of phase, and a significant interaction. Separate one-way ANOVAs indicated that in phase 1, the photorefractory birds differed significantly from the photosensitive and photostimulated groups; in phase 2, the photostimulated group no longer differed significantly from the photorefractory group, but the photosensitive group did.

effect of phase of the experiment ($F_{1,15}=22.3$, $P=0.006$) and a significant interaction between these two main effects ($F_{2,15}=7.24$, $P=0.006$). Scheffe's post hoc comparisons indicated that the photorefractory birds stored significantly less than the photosensitive and photostimulated birds ($P<0.05$) and that the mean seeds stored differed between phase 1 and phase 2 of the experiment.

To explore further the nature of the significant interaction term in the two-way ANOVA, we ran one-way ANOVAs comparing groups separately for phase 1 and phase 2. In phase 1, there was significant variation among groups ($F_{2,15}=7.37$, $P=0.006$) and Scheffe's post hoc comparisons indicated that the photorefractory birds stored significantly fewer seeds than the other two groups, which did not differ from each other. In phase 2, there was again significant variation between groups ($F_{2,15}=4.89$, $P=0.02$). However, Scheffe's post hoc comparisons indicated that the photostimulated group was no longer significantly different from the photorefractory group, whereas the photorefractory and photosensitive birds still differed significantly. Thus, we interpret the significant interaction term in the two-way ANOVA as indicating that the photostimulated birds changed storing between phase 1 and phase 2, but the other two groups did not. Further examination of individual storing sessions suggested that the rate of storing by the photostimulated birds dropped to the basal levels shown by photorefractory birds by the second storing session (after 4 days of photostimulation), although high variation precluded statistical analyses of the time course of this effect.

Hippocampus Volume

Among the birds used in this study, hippocampus volume varied independently of telencephalon size (simple linear regression: $r^2=0.023$, $N=18$). Inspection of

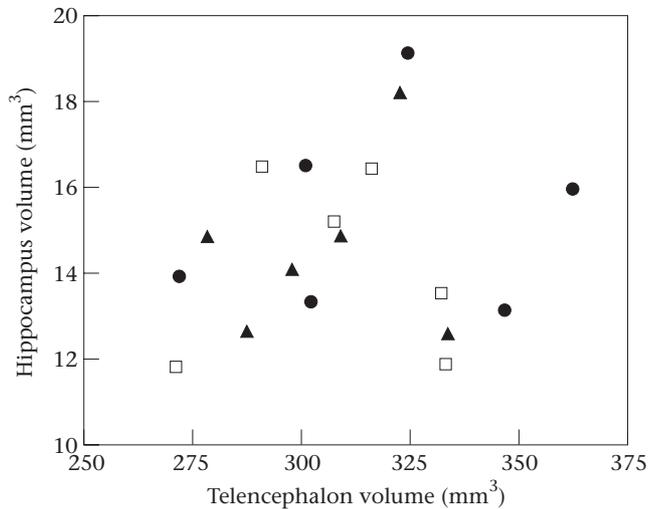


Figure 3. Plot of hippocampus volume versus telencephalon volume for photosensitive (□), photostimulated (▲) and photorefractory (●) black-capped chickadees.

Fig. 3 suggests a correlation between hippocampus and telencephalon volume for most birds, with the five birds having the largest telencephalon lying outside this relation. These five birds included members of all three treatment groups and both males and a female. We could find no variable (such as sex, time of capture, amount of storing) that might explain why these individuals had a large telencephalon but a relatively small hippocampus. Exclusion of these birds from further analyses did not alter the statistical results reported below.

There was no significant variation between groups in hippocampus volume (one-way ANOVA: $F_{2,15}=0.42$, $P=0.67$; Fig. 3). As reported in previous studies (Petersen & Sherry 1996), we also found no sex difference in hippocampus volume ($F_{1,16}=0.15$, $P=0.7$). There was no correlation between hippocampus volume and the mean number of seeds stored per session in phase 2 of the experiment (simple correlation: $r=-0.17$). Reanalysis of these data using an ANCOVA to account for variation in telencephalon size yielded identical results (that is, no effect of treatment group or telencephalon size on hippocampus volume). Thus, we found no effects of photoperiod treatment on hippocampus size.

We performed power analyses following Zar (1999) to determine whether this lack of effect on hippocampus size was merely a result of lack of statistical power. The variance in our data was similar to that in previous studies. For example, the standard deviations for adult hippocampus volume ranged from 1.34 to 2.92 mm³ in Smulders et al. (1995; $N=4-6$ birds per group) and were 2.3, 2.2 and 2.1 mm³ for the three groups in this study. Smulders et al. (1995) found that the hippocampus of birds caught in October was larger by 5.53 mm³ (or 34%) than the hippocampus of birds caught at other times of year. Based on the Pearson & Hartley (1951) power charts, we had a 97% chance of detecting such an effect size. Moreover, with 90% confidence we could have detected an effect size of 4.8 mm³ (33% difference in volume) and with 80% confidence we could have detected an effect

size of 4.1 mm³ (28% difference in volume, or 1.96 SD). Thus, we conclude that the statistical power in our analysis was more than sufficient to detect previously observed effect sizes.

DISCUSSION

Photoperiod and Food Storing

This study found that photoperiod appears to regulate food storing independently of photoperiodic state and gonad size. That is, birds on long days stored fewer seeds than birds on short days whether they were photostimulated, with large gonads and presumably high levels of plasma sex steroid hormones, or photorefractory, with small gonads and presumably low levels of plasma sex steroid hormones. This result suggests that the gonadotropic and sex steroid hormones that regulate seasonal changes in reproductive physiology and behaviour do not play a central role in regulating seasonal changes in food storing. Nongonadal steroids may still be involved, however, as has recently been suggested in the regulation of winter aggression by songbirds (Wingfield et al. 2001). Thus, further hormone manipulations will be required to further resolve this issue.

Candidates other than sex steroids that might regulate food storing include melatonin and/or thyroid hormones. Melatonin has recently been shown to play a role in seasonal changes in immune function and neural plasticity of song-control nuclei in European starlings, *Sturnus vulgaris* (reviewed in Bentley 2001). Furthermore, up-regulation of melatonin receptors in the song-control system in photorefractory starlings can occur without changes in photoperiod, sex steroids or melatonin and is most likely a more direct result of the state of photorefractoriness (Bentley & Ball 2000).

Although this study suggests that gonadotropins and gonadal steroids do not directly regulate food storing, it does not rule out the possibility that physiological state (photorefractory versus photosensitive) itself may affect food storing. Seasonal changes in the brain have been demonstrated in castrated songbirds showing that physiological state can affect brain and behaviour in the absence of gonadal steroids (e.g. Bernard et al. 1997). With respect to food-storing behaviour, it is unclear whether photorefractory birds on short days (a condition not examined in the present study) would store more or less than photosensitive birds on short days. It is likely that birds captured in the autumn were photorefractory, and when put on short days at the beginning of phase 1, these birds stored at high levels. Their photoperiodic state was not experimentally controlled, however, to ensure photorefractoriness.

Prior researches on photoperiod effects support the idea that both physiological state and photoperiod may affect food storing. Shettleworth et al. (1995) captured chickadees in March and November. Of the birds captured in March, half were kept on short days (and presumably were kept photosensitive long-term) and the other half were photostimulated for about 8 weeks, then returned to short days. Both groups stored little at first.

Eventually, when on short days, both groups began to store seeds, but the birds that had been photostimulated and had then become photorefractory and moulted, stored more than the photosensitive birds. The chickadees captured in November stored at high rates shortly following capture. Birds at this time were experiencing a short-day photoperiod, but it is not known whether they were photorefractory or photosensitive. Shettleworth et al. (1995) then photostimulated the birds in both groups, one shortly after capture, and one several months later. Both groups decreased storing, but at a slower rate than birds in our study. Shettleworth et al.'s results suggest that short days and physiological state interact to regulate food-storing behaviour, and are not inconsistent with the results found here.

Clayton & Cristol (1996) also reported an effect of photoperiod on food storing. They captured marsh tits, *Parus palustris*, in summer and transferred one group of birds to short days in September/October and then back to long days in November. A second group of birds were not transferred to short days until November. In both groups the birds stored more seeds when housed on short days than when housed on long days. The first group of birds began storing when transferred to short days, then reduced storing when transferred to long days. The second group did not start storing until they were transferred to short days. Unfortunately, the physiological state of these birds was unknown. In contrast to black-capped chickadees, neither group of marsh tits began feather moult (usually associated with photorefractoriness) until they were transferred to short days. Black-capped chickadees held on constant long days will become photorefractory and initiate feather moult without a reduction in photoperiod (S. A. MacDougall-Shackleton, unpublished data). Thus, photoperiod responses may differ between black-capped chickadees and marsh tits, so comparisons should be made cautiously.

This potential difference in photoperiod responsiveness between black-capped chickadees and marsh tits highlights an important limitation of this and other studies. The species of birds that have been most intensively studied in terms of photoperiodism (e.g. European starlings; white-crowned sparrows, *Zonotrichia leucophrys*; house finches, *Carpodacus mexicanus*, etc.) do not cache food. Reciprocally, there is very little information on the basic responses to photoperiod in food-storing species such as chickadees. Do chickadees need to experience short days to break photorefractoriness? What time of year do chickadees break refractoriness and how does this relate to an increase in food storing? Further studies on basic photoperiod responses in chickadees and other food-storing birds are required before we can fully understand the neuroendocrine bases of seasonal changes in food storing.

It is also possible that daylength has a proximate effect on food storing by a mechanism other than photoperiod-regulated hormones. For example, short days provide less time for feeding and impose a longer overnight fast. Food storing may be a direct response to the energetic consequences of shortened foraging time. However, it is not

clear whether a simple reduction in the availability of food causes an increase in food caching. Experiments that reduce the availability of food also make the occurrence of food less predictable (Lucas & Walter 1991; Hurly 1992; Lucas et al. 1993; Pravosudov & Grubb 1997; Pravosudov & Clayton 2001). Such manipulations may (e.g. Hurly 1992; Pravosudov & Grubb 1997) or may not (e.g. Pravosudov & Clayton 2001) increase the amount of food stored per day.

Seasonal Changes in Hippocampus Volume

Despite changes in food storing induced by changes in photoperiod, we did not observe any difference between our treatment groups in hippocampus volume. These results are consistent with those of Krebs et al. (1995), who replicated the photoperiod manipulation of Shettleworth et al. (1995). One group of birds was photostimulated, then transferred to short days at the beginning of moult, and a second group was held on short days long-term (photosensitive). Although the differences in food storing were identical to the results of Shettleworth et al. (1995), no difference was found in the volume of the hippocampus relative to the telencephalon.

Results that differ from those of Krebs et al. (1995) and those presented here were obtained in a field study of black-capped chickadees collected from the wild at different times of year (Smulders et al. 1995). Birds collected in October had a relatively larger hippocampal volume than birds collected at other times of year. October birds also had a greater total number of cells in the hippocampus (Smulders et al. 2000b). Barnea & Nottebohm (1994) had previously discovered that more new cells are incorporated into the hippocampus in October than at other times of year, although they did not find seasonal changes in total hippocampal neuron number.

Seasonal changes in hippocampus volume have been reported in cowbirds (Clayton et al. 1997). The hippocampus is larger during the breeding season in shiny cowbirds, *Molothrus bonarensis*, a generalist brood parasite. Female shiny cowbirds also have a larger hippocampus than males, but no interaction between sex and season was reported. Clayton et al. (1997) caution, however, that this apparent effect of breeding season on hippocampus size may be an artefact of histological processing of brains in different batches or confounding effects of different age distributions at different times of year. Seasonal changes in the hippocampus have also not been observed in food-storing squirrels (Lavenex et al. 2000a, b). Therefore, field data suggest a potential seasonal change in neuronal recruitment to the chickadee hippocampus, but the evidence from the field regarding seasonal volumetric changes in the vertebrate hippocampus is mixed.

The results of our study show, like previous laboratory results, no effect of photoperiod on hippocampal volume. Thus, laboratory photoperiod manipulations fail to replicate seasonal changes observed in the wild. Further research is needed to resolve this discrepancy. Increased neuron recruitment does not necessarily result in

increased volume, assuming that new neurons are predominantly replacing cells that have died (Barnea & Nottebohm 1994). The results obtained in the field by Smulders et al. (1995) showing seasonal change in hippocampal size in chickadees may be due to confounding factors that are difficult to control in field studies, as discussed by Krebs et al. (1995), and thus, the chickadee hippocampus may not undergo seasonal variation in size. Conversely, the chickadee hippocampus may vary in size seasonally as described by Smulders et al. (1995), but this effect does not occur under laboratory conditions, perhaps due to the effects of captivity; other songbird species show reduced hippocampus volume in captivity (Smulders et al. 2000a). Thus, diet, home range size, weather conditions, the level of storing and cache recovery, general activity and many other factors that differ between field and laboratory conditions may account for the difference between field and laboratory results. It is clear that if seasonal volumetric changes of the hippocampus do occur, they are far more subtle than other seasonal changes in the songbird brain such as the song-control system (reviewed in Ball 1999; Tramontin & Brenowitz 2000). Confirming the nature and mechanisms of seasonal plasticity in the hippocampus of food-storing birds will require greater integration of field and laboratory studies.

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