

Seasonal modulation of sickness behavior in free-living northwestern song sparrows (*Melospiza melodia morphna*)

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Summary

A variety of vertebrate species modulate immune function on a seasonal basis to cope with seasonal energy deficits and competing life-history demands, such as reproduction. Most studies to date have focused upon seasonal variation of cellular and humoral immunity, while neglecting behavioral responses to infection. These behavioral strategies are collectively termed sickness behaviors and are hypothesized to divert energy away from normal activities to combat and overcome infection. Sickness behavior can be triggered experimentally by injecting bacterial lipopolysaccharide (LPS). In this study, we provide the first evidence for seasonal modulation of sickness behavior in a free-living animal. Male song sparrows of western Washington state (*Melospiza melodia morphna*) are sedentary and territorial year round, except for a brief time during molt. Treatment with LPS decreased territorial aggressive behavior of males in the winter (nonbreeding), but not in the spring (breeding).

Subjects were recaptured approx. 25 h after treatment. Recaptured LPS males in the winter lost more body mass than saline-injected controls while LPS males in the spring did not. These data indicate that birds in breeding condition were relatively insensitive to the effects of LPS. On a proximate level, suppression of sickness behavior during breeding is likely mediated by seasonal differences in energy allocation, as wintering sparrows were significantly heavier and had larger subcutaneous fat reserves and lower baseline corticosterone levels than breeding birds. Ultimately, suppression of sickness behavior may represent an allocation strategy to balance current reproductive opportunities with the life-history costs of self-defense.

Key words: anorexia, bird, corticosterone, immune function, life history, lipopolysaccharide, seasonality, sickness behavior, territorial aggression.

Introduction

There is considerable evidence that immune function varies on a seasonal basis in a number of vertebrate taxa (Nelson and Demas, 1996; Nelson et al., 2002; Nelson, 2004), and several patterns of seasonal variation exist. Many animals that seasonally reproduce during the spring and summer exhibit reduced immune function in the winter when food is scarce and thermogenesis is increased to offset colder temperatures (Nelson and Demas, 1996; Nelson, 2004). This pattern is typically reversed in captivity, as short day lengths (non-breeding conditions) actually enhance immune function to anticipate these predictable energy deficits incurred during the winter months (Nelson and Demas, 1996). As a general rule, immune responses are energetically expensive to produce (Demas et al., 1997; Ots et al., 2001; Martin et al., 2003; Demas, 2004) and are tightly regulated by seasonal resource availability (Nelson, 2004), which is often lowest during the winter. Alternatively, immunosuppression can occur during the breeding season when investment in immune defense competes

with reproduction (Deerenberg et al., 1997; Nordling et al., 1998; Moreno et al., 1999).

The majority of studies examining seasonal variation of immunity have focused upon cellular and humoral components (Blom, 1994; Lochmiller et al., 1994; Demas and Nelson, 1996; Nelson and Demas, 1996; Zuk and Johnsen, 1998; Gonzalez et al., 1999; Moore and Siopes, 2000; Bilbo et al., 2002a; Møller et al., 2003; Nelson, 2004), while neglecting behavioral responses to infection. These behaviors are collectively referred to as sickness behaviors and include reduced activity, decreased food (anorexia) and water (adipsia) intake leading to weight loss, and a general decline in social activities and behavior (Hart, 1988). In concert with cytokine secretion from immune cells (e.g. interleukin-1), febrile responses, and glucocorticoid secretion, these behaviors encompass a unique ‘emergency life history stage’ (Wingfield, 2003), which is hypothesized to improve an organism’s ability to cope with infection by conserving energy for immune activation and reducing certain nutrients in the bloodstream, such as iron, that are essential for pathogenic growth and replication (Hart, 1988).

Despite these purported benefits, there are substantial energetic and life-history costs associated with sickness responses. Activation of sickness behavior accompanies the onset of the acute phase response, which is metabolically expensive to activate and maintain (Hart, 1988; Kluger et al., 1998a; Lochmiller and Deerenberg, 2000). Because food intake is suppressed, protein, lipid and carbohydrate stores are mobilized internally to fuel the energetic costs of inflammation and fever. In addition to these metabolic costs, expression of sickness behavior interferes with reproduction (Kent et al., 1992; Konsman et al., 2002) and associated activities such as territorial aggression and maintenance (Owen-Ashley et al., 2006), sexual behavior (Yirmiya et al., 1995) and parental care (Aubert et al., 1997). These reproductive activities are suppressed in favor of increased sleep for recruitment of additional resources to combat infection (Kent et al., 1992; Dantzer, 2001). Acute phase responses also proximately inhibit the hypothalamo-pituitary-gonadal axis (Rivier, 1990; Bosmann et al., 1996; Klein and Nelson, 1999), leading to a rapid decline in sex steroid levels. Furthermore, these life-history costs may persist until the animal has fully recovered from infection (Hart, 1988).

The seasonal modulation of behavioral immunity (i.e. sickness behavior) has been investigated in two studies to date involving captive animals, and both support a role for energy limitation. In male Siberian hamsters *Phodopus sungorus*, short day lengths reduce the duration of sickness behavior and fever following injection of lipopolysaccharide (LPS) compared to long-day (breeding) males (Bilbo et al., 2002b). LPS is an immunologically active component of gram-negative bacteria that induces acute phase responses and sickness behavior in vertebrates (Kent et al., 1992). Because hamsters experience significant reductions in body fat and mass after exposure to short day lengths, the attenuation of LPS-induced anorexia and fever is likely mediated by energy reserves. Similarly, male white-crowned sparrows (*Zonotrichia leucophrys gambelii*) held on short-day lengths exhibit diminished sickness responses (i.e. lose less mass) after LPS injection compared to males on long days, yet this seasonal effect is not observed in females (Owen-Ashley et al., 2006). These differences in sickness responses coincide with seasonal changes in body condition, as long-day males are heavier and have more fat reserves to lose than short-day males (Owen-Ashley et al., 2006). Interestingly, body condition in female white-crowned sparrows did not change according to photoperiod treatment, which agrees with sickness responses failing to vary seasonally (Owen-Ashley et al., 2006). These experiments support the hypothesis that modulation of sickness behavior is dependent upon a critical energy threshold: only individuals in good condition can afford to lose body mass to a minimum level, after which, subjects become relatively insensitive to LPS. Studies involving captive animals are instructive in providing general rules of thumb for predicting seasonal changes in sickness behavior, but it is unknown if this seasonal modulation would be readily translated to the wild, where resources are seasonally limited and unpredictable.

In this study, we examine if seasonal modulation of sickness behavior occurs in a free-living songbird, the rufous song

sparrow (*Melospiza melodia morphna*). This particular subspecies is sedentary and males defend territories throughout the year, except for a brief period during molt (August–September) when territorial aggressive behavior declines (Arcese, 1989; Wingfield, 1994). Breeding only occurs in the spring and summer (March–August), but males remain aggressive throughout the fall and winter if weather conditions are favorable (October–February) (Wingfield, 1994). Because LPS treatment reduces territorial aggression in wild male white-crowned sparrows (Owen-Ashley et al., 2006), we predicted that exposure to LPS in male song sparrows would also reduce territorial aggressive behavior both in the spring (breeding) and in the winter (non-breeding), but that modulation of the sickness response would be proximately affected by energy reserves. According to this hypothesis, birds in poorer condition with smaller fat reserves should become less sensitive towards LPS and experience a smaller reduction in territorial aggression than birds in good condition with larger reserves.

Materials and methods

Study sites

We treated free-living territorial male song sparrows *Melospiza melodia morphna* Oberholser 1899 with LPS or saline (control) and assessed aggression 24 h later in the breeding season (spring) of 2001 ($N=22$; 16–28 April) and in the non-breeding season (winter) of 2002 ($N=30$; 16 January–4 February). In each season, we captured birds at various localities in western Washington State: Friday Harbor laboratories (Global Positioning System coordinates: 48°33'N, 123°00'W) in the San Juan Islands [$N=4$, 4 (spring, winter)], Twin Harbors (46°51'N, 124°06'W; $N=3$, 6) and Ocean City State Parks (47°02'N, 124°09'W; $N=4$, 4) on the coast, and several sites along the Tolt River near Carnation (47°38'N, 121°55'W; $N=8$, 6). Additional field sites included Big Beef Creek Field Station (47°38'N, 122°46'W; $N=3$, spring) located on the Kitsap Peninsula, the University of Washington Experimental Pack Forest (46°50'N, 122°10'W) adjacent to Mt Rainier ($N=6$, winter) and Cedar Rock Preserve (48°33'N, 122°57'W) on Shaw Island in the San Juan Islands ($N=4$, winter). Treatment was balanced across sites except for odd sample sizes (N).

Initial capture and general field methods

Subjects were captured using mistnets and song playback. Immediately following capture (<3 min), a small blood sample (50–70 μ l) was collected from the alar wing vein for analysis of baseline corticosterone levels (see below). Blood was placed on ice, and then centrifuged to separate the plasma within 6 h. Plasma was stored at –20°C until conducting the radioimmunoassay (RIA; see below). Subjects were weighed (to the nearest 0.25 g) and scored for the extent of furcular and abdominal fat reserves using a semiquantitative scale (Helms and Drury, 1960; Wingfield and Farner, 1978). The two scores were then averaged for a cumulative measure of fat deposition. Males were banded with a numbered aluminum (National Biological Service) band on the left tarsus and a unique

combination of color bands on the right tarsus. All procedures were approved by the Animal Care Committee at the University of Washington under protocol #2212-31.

Within 5 min of capture, subjects were injected subcutaneously under the dorsal apertia immediately anterior to the right wing with 100 μ l of a water/oil emulsion that consisted of either LPS (Sigma#L4005, serotype 055:B5) dissolved in 0.9% saline (1 mg 1 ml⁻¹) and emulsified in a 1:1 ratio with Freund's Incomplete Adjuvant (FIA; Sigma#F5506) or 0.9% saline (control) emulsified with adjuvant (1:1 ratio). The specific dose was derived from dose-response studies in white-crowned sparrows (Owen-Ashley et al., 2006), a species similar in size and body mass to song sparrows. Emulsification of LPS with adjuvant was necessary to prolong the expression of sickness behavior past 24 h. Pilot experiments indicate that captive white-crowned sparrows administered LPS and Freund's incomplete adjuvant reduce food intake over a 48 h period, while birds treated with adjuvant alone experience a very slight decrease in food intake, but not a significant decline in body mass (see Owen-Ashley, 2004). Extending this response is important because behavior of male song sparrows was assessed 24 h after injection, and we wanted to ensure that subjects were still responding behaviorally to LPS. A colleague coded the treatments (A vs B), and assignment of treatment type alternated in sequence (A, B, A, B, etc.). The identity of the treatments was not revealed to the authors until the behavioral data were fully analyzed (see below). After injection, males were immediately released onto their territories.

Simulated territorial intrusion (STI)

Approximately 24 h later (± 10 min), we revisited territories and conducted a simulated territorial intrusion (STI) on responding males to assess territorial aggression (Wingfield, 1985). This technique involves placing a caged conspecific male on a focal male's territory along with a loudspeaker that broadcasts tape-recorded conspecific song, and then measuring territorial aggression. The playback sequence consisted of song variants that were repeated every 10–15 s for five repetitions. This sequence was followed by a 30 s interval of silence and then five repetitions of another song variant for a total of 15 different song types. Each song variant was previously recorded from different males to minimize pseudoreplication of playback stimuli (Kroodmsa, 1989). Multiple playback tapes were used so that birds initially captured using one type of playback stimulus were not exposed to the same song variants for the STI 24 h later. This was necessary to ensure that males were not associating particular song types with initial capture and handling. Although the duration of STI is typically 10 min (Wingfield, 1985; Wingfield and Hahn, 1994), we assessed aggressive behavior for 20 min because recently captured birds are generally more cautious compared to subjects revisited at a later date (e.g. 7–21 days) (Soma et al., 2000).

To quantify behavior, we assessed five behavioral measures that are typically associated with territorial aggression in male sparrows (Wingfield, 1994; Wingfield and Hahn, 1994): (1) the amount of time spent within 5 m of the decoy, (2) latency of

first response (s), (3) closest approach to the decoy (m), (4) the number of songs, and (5) the number of flights directed towards the decoy. We also measured the amount of time subjects were out of view, or hidden from the observer. This involved birds temporarily leaving the playback area (>30 m from decoy) or seeking refuge in bushes [a common behavior of sick birds (Owen-Ashley et al., 2006)]. STIs were not conducted in weather more severe than light, intermittent rain.

After the STI, the decoy was removed from the territory and the playback was stopped to assess the persistence of aggressive behavior for 10 min (Wingfield, 1994). The same behaviors described above were measured, except for latency of response. Behaviors were assessed relative to where the decoy cage used to be on the territory. Persistence of behavior after initial territorial intrusion plays an important role in the successful maintenance of a territory, especially for breeding males (Wingfield, 1994). For all trials, birds were observed through binoculars at a distance of 15–20 m relative to the decoy cage, and all observations were recorded into a handheld microcassette recorder. Some STIs were canceled due to adverse weather.

Recapture of birds

At the end of behavioral trials, we unfurled mist nets that had previously been erected before the start of STIs and resumed song playback. We allotted 60 min to attempt to recapture subjects. All recaptured birds were weighed and examined at the site of injection to determine if the adjuvant mixture was still present underneath the skin. The mixture is white, viscous and remains localized at the injection site, thus allowing for easy detection in recaptured birds. Subjects were then released immediately thereafter.

Radioimmunoassay (RIA)

Plasma corticosterone levels were assessed in a single direct RIA, as previously described (Wingfield et al., 1992). Intra-assay variability was 9.7%.

Statistical analysis

Fisher exact tests were employed to determine if the proportion of birds responding to STI and the proportion of those successfully recaptured were contingent upon treatment or season. Behavioral responses during and after STI were analyzed using separate MANOVAs, respectively, with treatment (LPS vs saline) and season (spring vs winter) as main effects. When appropriate, behavioral data were log-transformed to minimize deviations from normality and unequal variances (Zar, 1999). We used univariate tests (*t*-tests or Mann–Whitney tests) to examine specific within-season behavioral responses in relation to treatment. Retrospective power analyses were conducted for non-significant tests to ascertain minimum sample sizes necessary to achieve significance ($\alpha=0.05$; power=0.8).

Unpaired *t*-tests were used to compare initial body mass between seasons. Comparisons of body mass loss (%), fat score, and corticosterone levels were analyzed using

nonparametric Mann–Whitney tests. Significance was designated at $P < 0.05$.

Results

Territorial aggression

In the spring, nine out of 11 (82%) controls, and eight out of 11 (73%) LPS-injected birds were observed 24 h later and responded to the STI. In the winter, seven out of 15 (47%) controls and six out of 15 (40%) LPS-injected males were seen 24 h later and responded to the STI. The percentage of birds responding to STI was independent of season (Fisher exact test, $P = 0.15$) and treatment ($P = 0.78$).

Neither treatment (Wilk's $\lambda = 0.81$, $F_{6,21} = 0.80$, $P = 0.58$) nor season (Wilk's $\lambda = 0.65$, $F_{6,21} = 1.86$, $P = 0.14$) significantly affected male territorial aggressive behavior during STI. However, a significant interaction between treatment and season was detected (Wilk's $\lambda = 0.55$, $F_{6,21} = 2.9$, $P = 0.03$) with LPS treatment reducing male territorial aggressive behavior in response to STI in the winter (non-breeding), but not in the spring (breeding) (Fig. 1). For individual behaviors, LPS males in the winter spent significantly more time out of view than

saline-injected birds ($Z = -2.5$, $P = 0.012$). In addition, wintering LPS males spent less time within 5 m of the decoy (log-transformed, $t_{11} = -2.55$, $P = 0.027$) and produced fewer songs (log-transformed, $t_{11} = -2.40$, $P = 0.035$) compared to controls. Saline-treated males during the winter approached the decoy closer than LPS males ($t_{11} = 2.34$; $P = 0.039$). Males treated with LPS also tended to perform fewer flights towards the decoy than controls in winter ($t_{11} = -2.06$, $P = 0.06$). However, latency of first response in LPS males was not significantly different from controls in the winter ($Z = 1.14$, $P = 0.13$). In the spring, LPS males did not differ in any of the above behavioral measures relative to controls (all $P > 0.21$; Fig. 1). For these results, retrospective power analyses indicated that the minimum sample size necessary to achieve a significant effect ranged from 40–360 individuals. Because less than half of the birds originally captured responded to STI during the winter, this suggests that only the most aggressive birds were being assessed compared to birds in the spring. This may be one reason why several measures of aggressive behavior (time within 5 m of decoy, closest approach) seemed putatively higher in winter controls compared to breeding subjects.

Treatment also tended to influence aggressive behavior after the STI. Using MANOVA, the effect of treatment on the persistence of aggression approached significance (Wilk's $\lambda = 0.63$, $F_{5,22} = 2.6$, $P = 0.051$), with a tendency for reduced aggression in LPS males compared to controls. A significant effect of season was detected (Wilk's $\lambda = 0.59$, $F_{5,22} = 3.1$, $P = 0.029$) with males in the spring exhibiting overall greater levels of aggression after STI than males in winter (Fig. 2). However, there was no significant interaction between treatment and season (Wilk's $\lambda = 0.75$, $F_{5,22} = 1.5$, $P = 0.24$). In wintering birds, the closest approach was greater in LPS birds compared to controls (log-transformed, $t_{11} = 2.28$, $P = 0.02$), and there was a tendency for LPS males to spend less time within 5 m of the decoy than controls ($t_{11} = -2.09$, $P = 0.06$; Fig. 2). In contrast, no significant differences in individual measures of aggressive behavior were detected between LPS males and controls in the spring (all $P > 0.32$). For these results, the minimum sample size required to achieve significance ranged from 68 to 1280 using power analysis.

Recapture of birds and body mass loss

For males that responded to STI, we were able to recapture five LPS birds (63%) and four control birds (44%) in the spring, and four LPS birds (67%) and six control birds (86%) in the winter. Neither treatment (Fisher exact test, $P = 0.26$) nor season ($P = 0.99$) significantly biased recapture rates. These subjects were recaptured immediately following the behavioral trials (within 1 h). The Freund's incomplete adjuvant mixture (see Materials and methods) was present underneath the skin of all recaptured subjects.

Only males in winter experienced LPS-induced body mass loss 24 h after injection compared to controls (LPS vs SAL: spring, $Z = -0.3$, $P = 0.81$; winter, $Z = -2.6$, $P = 0.01$; Fig. 3). LPS males in winter lost, on average, $5.2 \pm 0.6\%$ of their body mass, while controls lost $0.74 \pm 1.05\%$ (Fig. 3). Males injected with

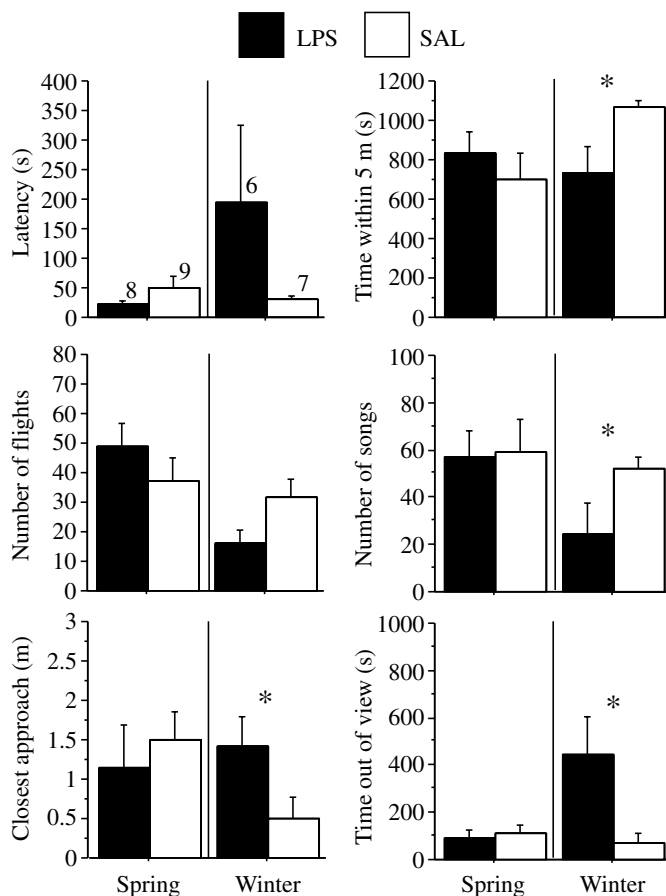


Fig. 1. Effect of lipopolysaccharide (LPS) or saline (SAL) treatment on aggression during simulated territorial intrusion (STI) in spring (breeding) and winter (nonbreeding). Values are means \pm s.e.m.; N, values are given in top left panel. * $P < 0.05$.

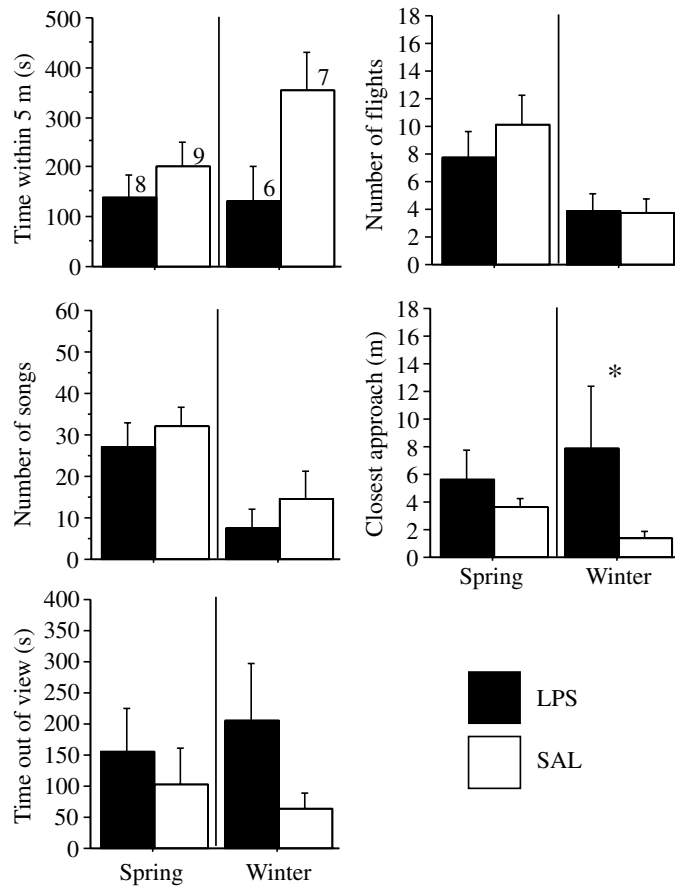


Fig. 2. Effect of lipopolysaccharide (LPS) or saline (SAL) treatment on aggression after stimulated territorial intrusion (STI) in spring (breeding) and winter (nonbreeding). Values are means \pm s.e.m.; N, values are given in top left panel. * $P < 0.05$.

LPS or saline in the spring lost, on average, $2.5 \pm 1.3\%$ and $1.6 \pm 1.7\%$ of their body mass, respectively (Fig. 3).

Body condition and baseline corticosterone levels

The seasonal modulation of sickness behavior coincided with seasonal differences in body mass, fat deposition, and baseline corticosterone levels in free-living males (Fig. 4). At

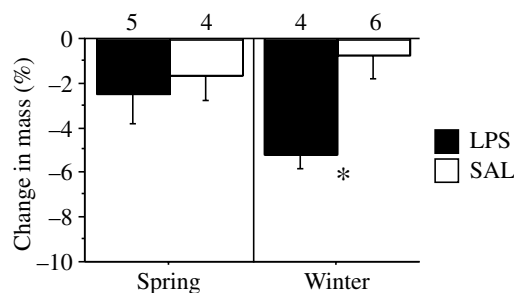


Fig. 3. Effect of lipopolysaccharide (LPS) or saline (SAL) treatment on the % change in body mass 24 h after injection. Values are means \pm s.e.m. (N values are given above bars). * $P < 0.05$.

initial capture, wintering males were significantly heavier (26.1 ± 0.3 g) and had larger fat depots (fat score: 1.8 ± 0.1) than males in the spring (mass: 24.7 ± 0.2 g; fat score: 0.3 ± 0.1 ; Spring vs Winter: body mass, $t_{50} = -3.98$, $P = 0.0002$; fat score, $Z = -5.98$, $P < 0.0001$; Fig. 4). Baseline corticosterone levels of males were significantly higher in the spring (16.79 ± 1.99 ng ml $^{-1}$) compared to males in the winter (11.16 ± 0.97 ng ml $^{-1}$; $Z = -2.12$, $P = 0.03$; Fig. 4).

Discussion

In free-living male song sparrows, we found evidence for seasonal modulation of LPS-induced sickness behavior. Several measures of territorial aggression during STI were reduced after LPS treatment in non-breeding birds compared to controls, but these effects were not detected respectively in breeding males. Furthermore, these differences in behavior were reflected in the overall MANOVA model, as indicated by a significant treatment by season interaction. In contrast, behavioral differences after STI were less pronounced, as MANOVA analyses failed to detect a similar interaction. However, a significant difference in one behavioral measure was identified in non-breeding males: non-breeding controls approached closer to the decoy site than LPS males. Birds exhibiting sickness behavior may be less inclined to approach an intruder on its territory, although it is unclear why other behaviors were not correspondingly affected.

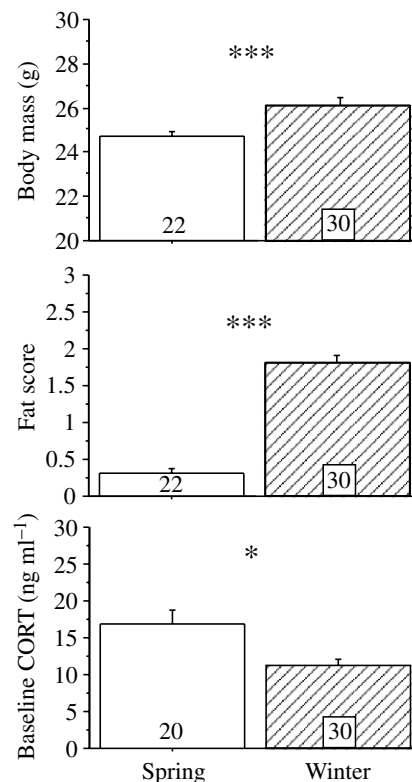


Fig. 4. Seasonal differences in body mass (g), fat depots, and baseline corticosterone levels (CORT; ng ml $^{-1}$) before injection. Values are means \pm s.e.m. (N values are given within bars). * $P < 0.05$, *** $P < 0.001$.

These behavioral differences mirrored the seasonal differences in body mass loss as measured in recaptured birds. Only non-breeding males injected with LPS experienced body mass loss 24 h following injection compared to controls. The decline in body mass is likely due to decreased feeding (anorexia) as a result of LPS-induced sickness. In contrast, males in breeding condition failed to exhibit reduced aggressive behavior or body mass loss after LPS treatment, indicating relative insensitivity to this particular dose of endotoxin.

This behavioral insensitivity was striking in relation to previous findings that a similar dose of LPS dramatically reduced territorial aggression in free-living white-crowned sparrows (*Z. l. gambelii*) during the breeding season in northern Alaska (Owen-Ashley et al., 2006). There are several possible reasons for this discrepancy. First, the stage of breeding differed between the two species. Territorial aggression in white-crowned sparrows was assessed when males were feeding nestlings, and thus investing little in territorial defense (although males still responded to simulated intrusion). In birds that breed in the Arctic and sub-Arctic regions, such as *Z. l. gambelii*, the transition between territorial and parental behavior is often abrupt, and aggressive behavior can decline rapidly (Wingfield and Hunt, 2002). In contrast, male song sparrows in April were aggressively defending territories, and pairs were initiating their first clutch of the season. Activation of sickness behavior during this time could be maladaptive if reduced territorial defense leads to loss of offspring (Avistur and Yirmiya, 1999). Second, the two species could differ in their sensitivity to LPS, such that male song sparrows require a higher dose to express sickness behavior. This hypothesis requires further examination, but sensitivity to LPS varies considerably in birds and mammals (Kluger et al., 1998b), even between closely related passerine species (e.g. Lee et al., 2005).

Whether breeding males were completely unresponsive to endotoxin challenge is equivocal, as LPS tended to decrease the persistence of aggressive behavior when both seasons were combined, although this result was not statistically significant. In addition, it is unlikely that subtle treatment effects in behavior could be detected in the spring given the low return rates of responding birds in general. Retrospective power analyses indicated that an order-of-magnitude increase in sample size would be necessary to detect any significant differences in individual aggressive behaviors for breeding males both during and after STI. Given these large projected sample sizes, these treatment effects are much more difficult to detect and may not be biologically significant compared to those documented in non-breeding males. Nonetheless, the specific finding that breeding males are completely insensitive to LPS warrants caution and further study.

Irrespective of treatment, breeding males were more aggressive overall after STI than non-breeding subjects, as previously described (Wingfield, 1994). During the breeding season, territorial male sparrows continue to patrol the territory and sing spontaneously for several hours after initial intrusion (Wingfield, 1994). This persistence is markedly reduced during the non-breeding season, when prolonged aggression may be

detrimental for males because days are short and time for feeding is abbreviated.

These findings are consistent with captive studies documenting that modulation of sickness behavior can occur on a seasonal basis. Male Siberian hamsters and male white-crowned sparrows exposed to long day lengths (breeding conditions) exhibit more robust sickness responses to LPS than males on short days (non-breeding) (Bilbo et al., 2002b; Owen-Ashley et al., 2006). However, it should be emphasized that the opposite effect occurred in this study: non-breeding males in the wild expressed sickness behavior, but breeding males failed to respond behaviorally to LPS. This contrary result is most likely tied to differences in the seasonal accumulation of energy reserves between captive white-crowned sparrows and free-living northwestern song sparrows, although there are other possibilities as well (see below).

We summarize five potential mechanisms that could contribute to the seasonal modulation of sickness behavior in free-living song sparrows: (1) energy limitation, (2) corticosterone, (3) testosterone, (4) social context, and (5) temperature. It should be noted that these mechanisms are not necessarily mutually exclusive and may compliment and interact with each other.

Energy limitation

Seasonal energy deficits dictate the partitioning of resources that can be allocated towards costly immune defenses and other life-history functions (Nelson, 2004). The LPS-induced sickness response creates an energetic bottleneck because individuals reduce their food intake at a time when the resource demands of the immune system are high. The energy limitation hypothesis posits that subjects can afford to maintain sickness behavior only for a fixed duration, after which any further decreases in energy stores from anorexia would be disadvantageous for survival (Bilbo et al., 2002b). In captive studies, long-day Gambel's white-crowned sparrows were in better condition (larger mass and greater fat stores) than short-day subjects (Owen-Ashley et al., 2006). This is because long-day photostimulation (>12 h) triggers the onset of pre-migratory hyperphagia, which increases body mass and fat deposition in preparation for the long vernal migration (King and Farner, 1963). In accordance with the energy limitation hypothesis, long-day males lost a greater percentage of body mass after LPS exposure compared to short-day birds. However, in free-living northwestern song sparrows, the reverse pattern occurred: males in the winter (non-breeding) were actually heavier and had larger fat depots than males in the spring (breeding), and these differences were highly significant. Thus, the opposing pattern in seasonal energy accumulation between captive and free-living birds predicts the observed differences in seasonal modulation of sickness behavior.

Fat deposits are a major contributor to the total energy balance of many vertebrates, and often vary on a seasonal basis. Winter fattening is a common life-history strategy employed by many temperate-zone birds to offset the longer periods of overnight fasting in the winter, and to provide emergency reserves during inclement weather or unpredictable food

shortages (McNamara and Houston, 1990). Therefore, male song sparrows may be able to afford to lose a portion of their body mass and fat stores after LPS injection, but only to a minimum threshold, after which birds must compensate and increase food and water intake. There is accumulating evidence that wintering birds manage their fat reserves at an optimum level rather than constantly maximizing their food intake, presumably to balance the risks of starvation and predation (McNamara and Houston, 1990; Gosler et al., 1995; Gentle and Gosler, 2001). Excessive fat reserves increase body mass, and thereby reduce agility and flight performance, thus imposing a survival cost when a bird tries to escape from a predator. Thus, this flexibility in regulating energy reserves during winter may permit birds to respond behaviorally to pathogenic infection without necessarily compromising survival.

Corticosterone

Baseline corticosterone levels were also elevated in breeding males, suggesting that energy turnover was occurring at higher rates than in winter, which could lead to faster metabolism and breakdown of LPS. It is well established that glucocorticosteroids promote energy mobilization (Munck et al., 1984; Wingfield and Silverin, 2002) and that baseline levels vary seasonally in many vertebrate species (Romero, 2002). Higher plasma levels of corticosterone during breeding may reflect increased energy costs associated with reproductive effort, such as gonadal development, territorial defense, and high testosterone levels (Bentley et al., 1998; Ketterson and Nolan, Jr, 1999; Martin et al., 2003; Greenman et al., 2005). These increased energy demands may compete with activation of LPS-induced sickness responses. Taken together, these data suggest that low energy availability in breeding males coincides with decreased sensitivity to infection, thus supporting the energy limitation hypothesis.

It is also feasible that the LPS-induced release of corticosterone could vary on a seasonal basis, which could indirectly suppress sickness behaviors through negative feedback of cytokine release and acute phase response activation (Munck et al., 1984; Besedovsky and del Ray, 1996; McEwen et al., 1997). Because we failed to measure glucocorticoid levels in recaptured subjects, this possibility cannot be excluded. In captive studies, seasonal modulation of adrenocortical responses to LPS does not occur in white-crowned sparrows (Owen-Ashley et al., 2006), but has been documented in Siberian hamsters (Bilbo et al., 2003).

Testosterone

An additional complimentary mechanism that could contribute to seasonal modulation of sickness behavior is the inhibitory role of testosterone on immune function. In general, testosterone suppresses acquired immune function (Grossman, 1984; Alexander and Stimson, 1988; Hillgarth and Wingfield, 1997) through direct activation of androgen receptors or through indirect mechanisms (Owen-Ashley, 2004). For example, testosterone implants in sparrows chronically increase plasma levels of glucocorticosteroids (Evans et al., 2000; Casto et al.,

2001; Owen-Ashley et al., 2004), which, in turn, may suppress or downregulate immune function (Munck et al., 1984; Apanius, 1998). More recent research indicates that testosterone treatment reduces the expression of sickness behavior in captive male white-crowned sparrows, although basal corticosterone levels are also elevated (Owen-Ashley, 2004). Whether the mechanisms responsible for suppression of acquired immune function by testosterone also apply to innate defenses, such as behavioral responses to infection, are unclear at this point.

Plasma testosterone levels in male song sparrows are basal in the fall and winter, but increase in early spring to peak levels in April during courtship and egg-laying that are coincident with heightened male-male territorial encounters (Wingfield, 1994). In seasonally breeding birds, androgen levels are reduced in captive males exposed to long daylengths (breeding conditions), presumably because appropriate social and environmental stimuli necessary to stimulate the reproductive axis and secretion of testosterone above a breeding baseline are lacking or insufficient (Wingfield and Silverin, 2002). Thus, in free-living birds, high circulating androgen levels during breeding could potentially mediate the behavioral insensitivity to LPS by regulating the suppression of sickness behavior.

Social context

Another possible explanation for modulation of sickness behavior is the effect of social context. Although the expression of aggressive behavior appears identical in breeding and non-breeding northwestern song sparrows, the context is not the same between seasons (Wingfield and Soma, 2002). During the spring, males aggressively defend breeding territories and mate-guard females from intruding males. During this time, defense of a breeding territory is integral to reproductive success (Arcese, 1989). Perhaps the presence of a fertile female would 'motivate' male song sparrows to suppress responses to infection if the costs of expressing sickness behavior lead to mate abandonment or loss of paternity (Avistur and Yirmiya, 1999). It should be noted that this suppression could potentially be regulated by testosterone, as the presence of a sexually receptive female is well known to elevate androgen levels in male songbirds (Moore, 1983).

During the non-breeding season, some males leave breeding territories and form 'alliances' with one or more birds to defend important food resources during the non-breeding season, although many pairs remain together on the same territory throughout the year (Wingfield and Monk, 1992). These non-breeding 'alliances' are only temporary, and some birds may switch territories completely or become floaters (Wingfield and Monk, 1992). Because of these alternative strategies, expression of sickness behavior during this time may be favored if territorial loss is less costly in the winter than in the spring.

Increasing evidence suggests that behavioral responses to infection represent a flexible, motivational state that can be modified according to both intrinsic and extrinsic factors (Aubert, 1999; Konsman et al., 2002). For instance, interleukin-1 treatment inhibits sexual behavior in female, but not male rats, while concomitantly decreasing locomotory activity in both sexes (Yirmiya et al., 1995). In microtine voles (*Microtus*),

males paired with females are less responsive to LPS challenge than males housed alone (Klein and Nelson, 1999). These sex differences may allow males to optimize reproductive potential, especially if mating opportunities are limited.

Temperature

A final mechanism that could alter expression of sickness behavior is ambient temperature. Because the initial host response to infection is energetically demanding (Lochmiller and Deerenberg, 2000; Nelson, 2004), animals should suppress immune function in colder environments if thermogenic demands are high. Blue tits (*Parus caeruleus*) exposed to low temperatures reduce antibody production to diphtheria–tetanus vaccination relative to birds at higher temperatures (Svensson et al., 1998), and deer mice (*Peromyscus maniculatus*) housed at 8°C decrease spleen mass and serum IgG levels compared to mice maintained at 20°C, regardless of photoperiod treatment (Demas and Nelson, 1996). Furthermore, lactating female rats reduce nest-building behavior and decrease activity after LPS injection at mild ambient temperatures. However, females cease to exhibit sickness behavior when exposed to cold temperatures that could potentially threaten survival of their pups (Aubert et al., 1997). These studies support the idea that suppression of sickness behavior should occur during winter, when temperatures are colder, instead of the spring. However, the relatively mild winters of the Pacific Northwest allow many songbirds to remain on their territories over the winter, instead of migrating south every year to warmer climates. Although ambient temperatures are still lower in the winter than in the spring in western Washington state, accumulation of fat reserves during winter may help alleviate this suppression of sickness behavior by serving as an energy reservoir if acute phase responses are activated.

Conclusions

These data provide the first evidence that songbirds seasonally modulate expression of sickness behavior in the wild. Males injected with the same dose of LPS expressed sickness behavior in the winter (nonbreeding), but no detectable sickness responses occurred in the spring (breeding). On a proximate level, we attributed this effect to seasonal differences in energy allocation, as non-breeding birds had more fat reserves available than breeding males that they could afford to lose. The high energetic costs of acute phase responses that accompany behavioral responses to infection are inextricably linked to the condition of the animal. If energy reserves are minimal, the marginal benefit accrued from exhibiting sickness behavior precipitously declines, as any further decreases in body mass below a critical set point may lead to a reduced chance for recovery (Bilbo et al., 2002b; Nelson et al., 2002). Thus, depending upon energy demands over the season, suppression of sickness behavior, and immune function in general, may actually be favored. Ultimately, inhibition of the sickness response during breeding may be an adaptive host strategy to balance competing reproductive and

survival functions. Future studies should investigate the long-term costs and benefits of expressing sickness behavior in the wild in relation to fecundity and survivorship.

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