

Swainson's thrushes in migratory disposition exhibit reduced immune function

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Abstract Evidence indicates that the immune system, which protects an organism from parasitic and pathogenic infections, is frequently suppressed when animals are engaged in activities involving strenuous exercise. We tested the hypothesis that birds reduce immune function during the migratory period in preparation for the anticipated heightened energetic demands of long flights. Swainson's thrushes (*Catharus ustulatus*), captured in fall, were held in an indoor aviary until January, when migratory disposition was induced in half of the birds with an artificially prolonged daylength. Experimental birds became hyperphagic and deposited fat stores, and then displayed nocturnal activity (*Zugunruhe*) characteristic of the spring migratory period. Cell-mediated immunity was measured by intradermal injection of phytohemagglutinin in the wing patagium of both control and experimental birds. Birds exhibiting migratory restlessness had a reduced cell-mediated immune response compared to control birds. Our results suggest that birds are immunosuppressed during the migratory period. The suppression may be a nonadaptive response due to unrelated physiological processes, or it may be an adaptive response to the physiological demands associated with migration, such as high energetic demands and the negative consequences of a hyperactive immune system.

Keywords Swainson's thrush · *Catharus ustulatus* · Migratory behavior · Phytohemagglutinin · Immune response

Introduction

The immune system protects an organism from parasitic and pathogenic infections. Yet evidence indicates that immune function is frequently suppressed when animals are engaged in energetically demanding activities (Norris and Evans 2000). Immunosuppression in response to energetically demanding activities may reflect a reallocation of energy. Such a trade-off rests on the assumption that maintaining and activating the immune system has nutritional or energetic costs (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000), that resources are limiting and that immune system activation and maintenance comes at the cost of resource allocation to other costly activities. Alternatively, immunosuppression during a resource-demanding and stressful period may be an adaptive response aimed at avoiding hyperactivation of the immune system and the ensuing autoimmune response (see Råberg et al. 1998).

Recent attention has focused on reproductive activities and their impact on the avian immune system (Sheldon and Verhulst 1996; Råberg et al. 2000). These studies demonstrate that animals typically reduce immune activity during the breeding season compared to other periods of the annual cycle (Ots and Hōrak 1996; Deerenberg et al. 1997; Lifjeld et al. 2002; Ilmonen et al. 2003; but see Hasselquist et al. 1999). Despite the energetic and other demands associated with migration, few studies have investigated the immune function of birds during the migration period (but see Ward and D'Cruz 1968; Silverin et al. 1999; Owen and Moore 2006; Hasselquist et al. 2007).

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In anticipation of the energy requirements of migration, especially in relation to non-stop flights across ecological barriers such as the Gulf of Mexico, a bird in migratory disposition becomes hyperphagic, depositing up to 50% of its lean body mass as fat within a few days (Berthold 1975). Fat is the primary source of energy to fuel migration (Blem 1990) and is typically depleted during long-distance flights (Bairlein 1985; Moore and Kerlinger 1987). In addition, body composition is often altered to facilitate migratory flight (Jehl 1997; Piersma et al. 1999; Landys-Ciannelli et al. 2003). Flight muscles typically increase in size, while nonessential tissues and organs atrophy.

Likewise, we suggest that birds may suppress immune function to assist in meeting the demands associated with the migratory period. If true, individuals in migratory disposition should have a lower immune response than nonmigratory individuals. Alternatively, birds may have enhanced immunocompetence during the migratory period to protect themselves from increased exposure to novel pathogens and parasites (Moller and Erritzoe 1998; Hasselquist et al. 2007). Regardless, it is possible to study the immune response of migratory birds under controlled, experimental conditions because migratory disposition, including migratory activity [*zugunruhe*] and hyperphagia, can be induced in captive individuals by manipulating photoperiod (Berthold 1990). This induced activity corresponds approximately to the daily and annual pattern of migration of free-ranging conspecifics (Berthold 1990). We induced spring (return) migratory disposition in captive Swainson's thrush (*Catharus ustulatus*) by adjusting photoperiod. Swainson's thrushes are long-distance, intercontinental migrants that breed in the northern United States and Canada and winter in Central and South America (Evans and Yong 2000). Swainson's thrushes are trans-Gulf migrants with peak passage on the northern coast of the Gulf of Mexico in mid-late April (Yong and Moore 1997).

Materials and methods

During fall migration (October 2000) we captured 18 hatch-year Swainson's thrushes at our study site on the Fort Morgan Peninsula, Alabama, USA. Birds were randomly assigned to one of two rooms within an enclosed aviary located within the University Animal Research Facility [federal permit #21221, IACUC protocol #217-003]. We housed birds individually in cages equipped with two horizontal perches attached to microswitches, which recorded migratory activity via a data logger (JoAC Elektronik, Lund, Sweden). To minimize the effect of nutritional status on immune activity, both experimental and control birds were fed ad libitum a mixed diet of meal worms, blueberries, and moistened ZuPreem monkey

biscuits. We maintained birds on a nonmigratory 12:12 light:dark (L:D) photoperiod from day of capture until 31 January 2001, when we increased the photoperiod of treatment birds to a 16:8 L:D migratory schedule over a four-day period. We maintained control birds on a nonmigratory 12:12 L:D schedule throughout the experiment.

We considered birds as migratory when they exhibited migratory restlessness for longer than 40% of the night and for more than two nights in a row. This criterion is based on data collected on the nighttime activity of free-living, migrating Swainson's thrushes captured and held overnight in activity cages (J.C. Owen, unpublished data). We assessed cell-mediated immune function using phytohemagglutinin (PHA), an antigen that induces a nonspecific T-lymphocyte-dependent response (Stadecker et al. 1977). PHA induces dermal infiltration of leukocytes, macrophages and thrombocytes, causing increased swelling of the tissue at the inoculation site (Stadecker et al. 1977; Goto et al. 1978; McCorkle et al. 1980; Martin et al. 2006). Thus, the amount of swelling reflects the strength of an unspecific cell-mediated immune response. After treatment birds exhibited migratory restlessness for approximately two weeks, we administered (20 February 2001) an intradermal injection of PHA to both control ($n = 9$) and treatment birds ($n = 9$).

Prior to injection, we measured the right and left wing webs of the thrushes to the nearest 0.01 mm using a pressure-sensitive micrometer. We measured each wing web three times, averaging the measurements. We injected phosphate-buffered saline (PBS; 0.05 ml) into the right wing web, as a control, and injected PHA (0.25 mg/0.05 ml PBS) into the left wing web. Twenty-four hours post-injection, we remeasured each wing web without knowledge of previous measurements. We calculated the strength of the immune response by subtracting the change in thickness for the right wing web (pre- to post-treatment thickness) from the change in thickness in the left wing web (Goto et al. 1978; McCorkle et al. 1980).

We quantified the amount of migratory activity by dividing the number of 20-min nighttime periods in which each bird displayed at least five hops by the total number of nighttime periods available (treatment = 48 periods; control = 36 periods). To ensure that birds did not differ in migratory activity prior to the change in photoperiod, we compared the proportion of nighttime activity between the two groups using a Mann-Whitney test. After the change in photoperiod, we conducted the same analysis to detect differences in activity between the treatment and control groups.

We excluded one individual from each group; one bird in the control group displayed nighttime activity throughout the entire experiment, and one treatment bird was excluded because there was leakage of PHA at the time of

injection. All analyses are with the 16 remaining individuals. Immune response to PHA was analyzed using a two-tailed *t*-test. Data met assumptions of normality, and statistical significance was set at $\alpha = 0.05$. Data were analyzed using SPSS 12.0 (SPSS 2004).

Results

All of the thrushes in the treatment group exhibited migratory activity consistent with our a priori criteria. As found in other species (J.C. Owen, unpublished data), the Swainson's thrushes exhibit activity consistent with migration for several nights in a row and then reduce or suspend activity for one or two nights. The nights in which individuals reduced their activity were not the same for all individuals explaining the nightly variation (see Fig. 1). On a typical night, the number of treatment birds displaying "migratory" (active >40% of night) activity was 5–9 (mean = 6.9) of the nine individuals. In the control group, the number of birds meeting criteria for migratory activity ranged between 0 and 4 (mean = 2.7) of the nine individuals. However, none of the control birds showed consistent night-to-night activity that met the criteria.

In the 12 days prior to the change in photoperiod, we found no significant difference in nighttime activity between the treatment (mean rank = 13.29, $n = 12$) and control (mean rank = 11.79, $n = 12$) groups ($z = -0.492$, $p = 0.630$). In the 22 days following the increase in day-length, the migratory birds (M rank = 31.07, $n = 22$) showed greater nighttime activity than the nonmigratory

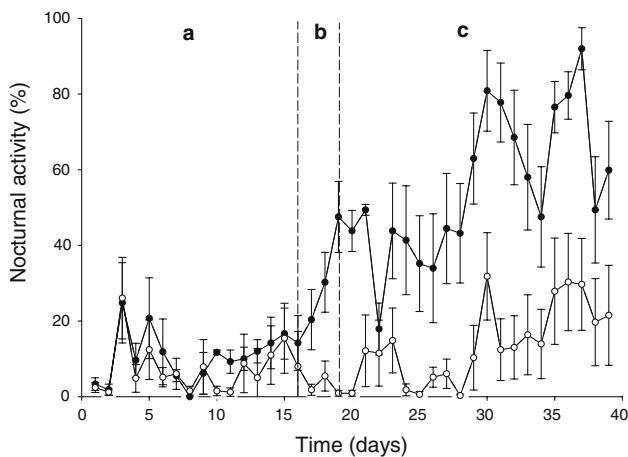


Fig. 1 Percentage (mean + SE) of the nighttime that Swainson's thrushes exhibited migratory activity. Closed circles, experimental birds; open circles, control birds. Time periods: a, when both groups were at a 12:12 light:dark (L:D) photoperiod; b, experimental birds were photoadvanced 1 h each day to induce migratory disposition; c, experimental birds were at a 16:8 L:D and control birds were at a 12:12 L:D photoperiod. All birds received phytohemagglutinin injection on day 37

birds (M rank = 13.93, $n = 22$) $z = -4.47$, $p < 0.001$ (Fig. 1). Thrushes displaying migratory activity had significantly lower PHA responses than the birds not in migratory disposition ($t = 2.148$, $p = 0.05$, $df = 14$; Fig. 2).

Discussion

Our results suggest that Swainson's thrushes exhibiting migratory restlessness may suppress their nonspecific cell-mediated immune response. This reduction in immune activity is consistent with studies which show that birds have smaller spleens (Fänge and Silverin 1985; Silverin et al. 1999; Deerenberg et al. 2003; Muñoz and De la Fuente 2003) and lower concentrations of leukocytes, particularly lymphocytes (Owen and Moore 2006) during the migratory period compared to other periods of the annual cycle. The avian spleen is a secondary immune organ, which plays an important role in disease resistance. It is one of the major sites of production, differentiation, and storage of lymphocytes, including those responsible for cell-mediated immune responses (Glick 2000). Therefore, the size of the spleen is thought to positively reflect a birds' ability to mount an immune response (John 1994); but see (Smith and Hunt 2004). In a comparable study, Gylfe et al. (2000) showed that redwing thrushes (*Turdus iliacus*) displaying migratory restlessness were more likely to exhibit a reactivation of a latent infection of *Borrelia burgdorferi*, the bacteria that causes Lyme's disease. Presumably, the reactivation was caused by a reduction in immune function.

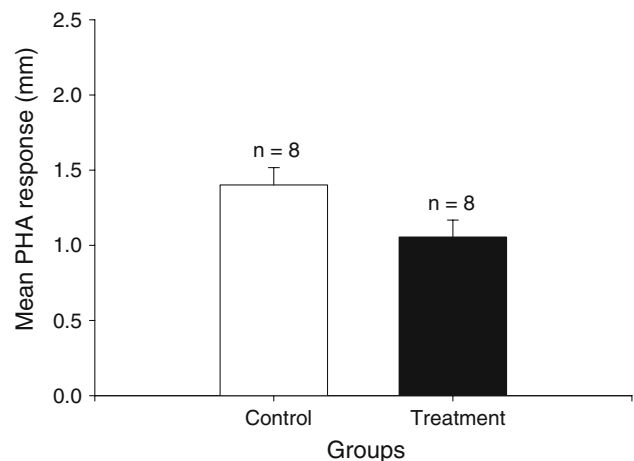


Fig. 2 Phytohemagglutinin (PHA) responses for nonmigratory (white bar) and migratory (black bar) Swainson's thrushes. Birds displaying migratory activity had reduced immune responses to PHA compared to nonmigrating individuals (*t*-test, $t = 2.148$, $df = 14$, $p = 0.05$). Bars represent mean PHA response + SE. Statistical significance is based on a Student's *t*-test (two-tailed)

Immunosuppression in migrating birds may be a consequence of unrelated physiological processes that cause fluctuations in the immune system (i.e., a nonadaptive response). On the other hand, migration is a predictable event in a bird's annual cycle, and suppressing the immune system may be an adaptation aimed at either allocating more energy toward other, more critical activities (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Norris and Evans 2000) or at preventing the damaging effects of an overactive immune response (Råberg et al. 1998). Here we explore these two possibilities.

Nonadaptive immunosuppression

The observed immunosuppression may be a consequence of a rise in reactive oxygen species (free radicals; ROS) during strenuous exercise. Increased oxygen consumption of working muscles generates ROS, which subsequently cause significant cellular damage (oxidative stress) to nucleic acids, lipids, and proteins, including those of the immune system (Niess et al. 1999). Another direct consequence of strenuous exercise and increased muscle use is inflammation. An inflammatory response is characterized by the proliferation and activation of several lines of immune system cells, such as neutrophils (the mammalian equivalent to avian heterophil), monocytes, and macrophages, which also generate ROS (Tidball 2005). In both cases, the ROS attack cells of the immune system and may be responsible for a reduced T cell response observed in migrating birds. Prolonged, intense exercise is characteristic of some migratory flights, and muscle damage has been observed in birds following such flights (Guglielmo et al. 2001). However, the current study was conducted with captive migrants, and we argue that *zugunruhe* did not impose significant energetic costs. Likewise, Hasselquist et al. (2007) found that red knots (*Calidris canutus*) engaging in long flights in a wind tunnel did not have reduced humoral and cell-mediated immune responses when compared to non-flying controls. Therefore, such strenuous exercise is an unlikely explanation for our results.

Another nonadaptive explanation for the observed immunosuppression may be linked to the action of melatonin, a primary hormone of the pineal gland, which mediates the bird's response to photoperiod. The amount of melatonin released is inversely related to day length. Evidence suggests that melatonin enhances immune function, especially in mammals (Nelson and Demas 1996). However, its role in avian immune function is not clear (Bentley et al. 1998; Moore and Siopes 2000; Haldar and Singh 2001). Japanese quail exposed to long days had reduced immune function, as measured by humoral and cell-mediated immune responses (Moore and Siopes 2000).

However, there was only a significant photoperiod effect in birds exposed to 24 h of light. The cell-mediated and humoral immune responses did not differ between 8:16 LD and the 16:8 LD groups, although melatonin levels were likely higher in the former. In our study, the difference in photoperiod is far less pronounced.

Baseline level of corticosterone, the primary glucocorticoid in birds, is elevated during the migratory period and is thought to facilitate hyperphagia and lipogenesis in relation to the energetic requirements of migration (Sapolsky et al. 2000). Glucocorticoids are also considered immunosuppressants, such that glucocorticoids inhibit cytokine activity, activation and proliferation of T cells (and to a lesser extent B cells), and the production and function of peripheral leukocytes (except for neutrophils) (Sapolsky et al. 2000). Therefore, the consequence of elevated baseline glucocorticoids during migration may be reduced immune function. While we did not measure corticosterone, there is evidence that baseline corticosterone levels rise in captive birds in response to photoperiod changes (Holberton 1999).

Adaptive immunosuppression

Immunosuppression in migrating birds may be an adaptive response aimed at enhancing the probability of a successful migration. For instance, suppression of the immune system may represent a trade-off in response to the energetic costs associated with the migratory period (Norris and Evans 2000). There is some evidence, albeit controversial, that the immune system is energetically costly to deploy (Klasing and Leshchinsky 1998; Ots et al. 2001; Martin et al. 2003, but see Svensson et al. 1998, Nilsson et al. 2007) and maintain (Lochmiller and Deerenberg 2000). Therefore, individuals that downregulate immune function and reallocate energy toward migration may gain an advantage. Animals engaging in intense, prolonged bouts of exercise exhibit reduced immune function (Hoffman-Goetz and Pedersen 1994; Pedersen et al. 1997; Råberg et al. 2000; Peijie et al. 2003; but see Hasselquist et al. 2007), with this immunosuppressive effect further exacerbated when the organism is food-deprived (Boyum et al. 1996).

Alternatively, immune function may be downregulated by neuroendocrine mechanisms in order to avoid immunopathology during periods of stress (Råberg et al. 1998). Strenuous exercise induces an inflammatory response, which is characterized by a rise in phagocytic cells and proinflammatory cytokines (Tidball 2005). The consequence of an unchecked inflammatory response is significant tissue damage. Therefore, an increase in circulating plasma corticosterone prior to strenuous exercise may prevent "overshooting" of the immune system and protect the organism from the subsequent immunopathology (Sapolsky

et al. 2000). Consequently, corticosterone may have a dual function in migrating birds; to facilitate fattening and to prevent overactive immune responses during prolonged flights.

Regardless of the mechanism, Swainson's thrushes exhibiting migratory restlessness had a reduced immune response to PHA. Our findings appear counter to the expectation that migratory species invest more resources in immune defense because migrants are exposed to more parasites and pathogens by virtue of their migratory life cycle (sensu Moller and Erritzoe 1998). However, it may be that migrating birds have a more robust immune system to begin with and they can thus afford to downregulate it without severely compromising overall disease resistance. For instance, human athletes have more active natural killer cells (Nieman et al. 1995, 2000) and higher PHA proliferation responses at rest than nonathletes (Nieman et al. 2000). Natural killer cells are critical in the defense against viruses, and their increased activity in resting athletes may help offset the immunosuppression that occurs during intensive exercise. Hence, the reduced immune response in migrating Swainson's thrushes may not increase susceptibility to disease en route.

More studies are needed to determine the functional significance of reduced immune function during the migratory period (Viney et al. 2005). During the nonmigratory period there is evidence that even a slight reduction in nonspecific immune response may translate into a decreased survival (Møller and Saino 2004). A reduction in immune function during the migratory period may have negative fitness consequences; an immunocompromised bird may be more likely to be infected with a parasite or pathogen, or may experience recrudescence of a latent infection during the migratory period.

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