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Effect of Sleep Loss on Executive Function and Baseline Corticosterone Levels in an Arctic-Breeding Songbird, the Lapland Longspur (Calcarius Lapponicus)

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EFFECT OF SLEEP LOSS ON EXECUTIVE FUNCTION AND BASELINE CORTICOSTERONE LEVELS IN AN ARCTIC-BREEDING SONGBIRD, THE LAPLAND LONGSPUR (CALCARIUS LAPPONICUS)

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By
Brett Hodinka

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EFFECT OF SLEEP LOSS ON EXECUTIVE FUNCTION AND BASELINE CORTICOSTERONE LEVELS IN AN ARCTIC-BREEDING SONGBIRD, THE LAPLAND LONGSPUR (CALCARIUS LAPPONICUS)

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I dedicate this thesis to Richard L. Hodinka, Ph.D.
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Sleep is a fundamental and essential component of vertebrate life, although its exact function remains unknown. Animals that are deprived of sleep typically show reduced neurobiological performance, health, and in some cases, survival. However, a number of animals exhibit adaptations that permit them to carry out normal activities even when sleep is restricted or deprived. Lapland longspurs (Calcarius lapponicus), arctic-breeding passerine birds, exhibit around-the-clock activity during their short breeding season, with an inactive period of only 3–4 h/day (71°N). Whether these birds suffer behavioral and physiological costs associated with acute sleep loss (SL) is unknown. To assess the effects of SL, wild-caught male longspurs were placed in captivity (12L:12D) and trained for 2 months using a series of memory tests, including color association and spatial learning to assess executive function. Birds were then placed in automated sleep fragmentation cages that utilize a moving wire to force movement every 1 min (60 arousals/h) during 12D (inactive period) or control conditions (during 12L; active period). After a single round of SL (or control) treatment, color association and spatial learning tests were conducted. Baseline plasma corticosterone concentration, body mass, and satiety were also assessed. SL significantly elevated corticosterone levels and increased accuracy during the color association test, but not the overall time required to complete the test. SL had no effect upon spatial
learning, body mass, or satiety. Taken together, these results suggest that Lapland longspurs exhibit a behavioral, but not a physiological, resilience to acute SL.
INTRODUCTION

Sleep is a fundamental and essential component of vertebrate life and is generally defined as a rapidly reversible state of reduced responsiveness to external stimulation, reduced motor activity, and reduced metabolism (Siegel, 2009; Chokroverty, 2017). However, the precise function of sleep has produced considerable debate. Although sleeping accounts for nearly one-third of a human’s life, the benefits associated with the recommended 7–9 h of sleep per night (Hirshkowitz et al., 2015) can be assessed only when sleep is prevented. Moreover, mounting evidence suggests that sleep is critical to learning and memory (Stickgold, 2005; Diekelmann and Born, 2010), and when sleep is disrupted, memory processing functions consequently diminish (Walker and Stickgold, 2006; Goel et al., 2009). In mice for example, studies found that disrupting sleep can significantly decrease performance on a series of executive function tests such as novel object recognition (Palchykova et al., 2006; Rolls et al., 2011) and food-motivated reaching (Vyazovskiy et al., 2011). In humans, significantly decreased reaction times on psychomotor vigilance tasks (Kribbs and Dinges, 1994) as well as deteriorated decision-making skills (Harrison and Horne, 2000) have been observed.

The detrimental effects associated with sleep, however, do not hold true when applied to other species throughout the animal kingdom. For example, although some insects display periods of inactivity, they fail to meet the behavioral definition of sleep despite having functional nervous systems (Siegel, 2008). Among mammals, time spent sleeping is quite variable, ranging from fewer than 3 h (e.g., elephants, giraffes) to more than 20 h per day (e.g., some bat species, opossum) (Zepelin et al., 2005; Siegel, 2009). The walrus undergoes a period of extended wakefulness for several days (Pryaslova et al.,
while dolphin and whales undergo unihemispheric slow-wave sleep (Lyamin et al., 2004) in which the other hemisphere of the brain displays electrophysiological correlates of wakefulness.

Birds are of particular interest when investigating the detrimental effects associated with sleep loss due to their extreme variability in behavioral and physiological responses to sleep loss (SL). Some species of birds respond more to SL while others are remarkably resilient during certain times of the year when sustained wakefulness is presumably needed (Zepelin et al., 2005; Fuchs et al., 2006). For example, white-crowned sparrows (Zonotrichia leucophyrys gambelii) reduce average sleep duration by 60% during migration compared with non-migratory states, but no deficits in learning or performance are documented relative to non-migratory stages (Rattenborg et al., 2004; Jones et al., 2010).

In addition to migratory sleeplessness, organisms inhabiting polar regions are inevitably exposed to extreme and unpredictable environmental conditions that may disrupt normal sleeping patterns. Around the summer and winter solstices, animals that reside in Antarctica or north of the Arctic circle (> 66°33’)) experience weeks to months of either continuous daylight or darkness, respectively (Pielou, 1994). For humans, constant light during the summer, as well as constant darkness in the winter, can alter sleep patterns and disrupt the circadian clock (Arendt, 2012). In a study by Lesku et al. (2012), polygynous pectoral sandpipers (Calidris melanotos) that breed in the Arctic exhibited “around-the-clock” activity that corresponds with high electromyogram activity and wakefulness. They proposed that intense competition for mates leads to “reproductive
sleeplessness,” as evidenced by the most active males siring a greater number of offspring (Lesku et al., 2012).

The family Calcariidae is an avian clade that includes arctic-breeding specialists with circumpolar distributions (Hussell and Montgomerie, 2002; Montgomerie and Lyon, 2011), such as the Lapland longspur (*Calcarius lapponicus*) and snow bunting (*Plectrophenax nivalis*). Due to the short summer season at high-latitudes, breeding is restricted to 5-6 weeks and is highly synchronized. Within this short season, pairs must be successful in accomplishing a number of breeding substages such as territory establishment, pair bonding, nesting, and parental care. Pairs only have time to produce one clutch, and re-nesting is only possible early in the season (Pielou, 1994). Any failure or disruption to one of these substages can reduce annual reproductive success to zero (Wingfield et al., 2004). A previous study indicated that male Lapland longspurs on their breeding grounds in Barrow, Alaska (71°N) spent approximately 20 h per day exhibiting behaviors associated with activity across a 24-h polar day (Ashley et al., 2013). If longspurs exhibited impairments associated with SL similar to those seen in other organisms, it would be expected that these birds would experience severe disadvantages on their breeding grounds where they compete for territory, resources, and mates. Therefore, the question remains: how and why are these species able to withstand the effects of sleep loss while other species (e.g., humans) are not as resilient?

Many birds in energetically demanding conditions exhibit elevated levels of corticosterone, a steroid hormone produced from the adrenal gland (Wingfield et al., 1997). Previous studies on mountain chickadees (*Poecile gambeli*) (Pravosodov et al., 2003; Pravosodov and Clayton, 2001) have shown that elevated levels of corticosterone
improve cache retrieval and increase accuracy on spatial memory tasks, but not nonspatial tests. In addition, zebra finch (Cooper et al., 2019) and mice (Dumaine and Ashley, 2015) increase basal levels of plasma corticosterone in response to SL. Thus, it is possible that the SL-induced elevation in glucocorticoids could play a role in mediating executive function performance in the Lapland longspur. Notably, other studies have shown that some alpine and arctic-breeders such as the white-crowned sparrow and dark-eyed junco (Junco hyemalis) have reduced adrenocortical responses and release less corticosterone in response to stressors (Bears et al., 2003; Wingfield et al., 1995). Thus, it is possible that the Lapland longspur may instead exhibit a physiological resilience to SL.

The aim of this study was to investigate whether experimental acute SL in an arctic-breeding passerine songbird, the Lapland longspur, exacts a physiological cost such as increased plasma corticosterone concentrations and/or a behavioral cost such as decreased cognitive performance on a series of executive function tests. Given the around-the-clock activity of free-living longspurs, I predicted that 12 h of SL would result in similar cognitive performance and no elevation in plasma corticosterone concentration when compared to controls. The alternative hypothesis is that 12 h of SL does significantly increase corticosterone concentrations and/or decrease cognitive performance relative to controls, suggesting that longspurs are sensitive to the effects of SL.

MATERIALS AND METHODS

Animals

Male Lapland longspurs (n = 29) were caught in Barrow, Alaska (71°32’ N, 156°67’ W) in June of 2018 using a combination of walk-in Potter traps, song playback, and live male decoys. Lapland longspurs are sexually dimorphic in plumage
characteristics during the breeding season (Hussell and Montgomerie, 2002) allowing sex identification upon capture. Birds were temporarily housed in an outdoor aviary at the Barrow Arctic Research Center until being placed in a modified pet carrier and transported by airplane to Western Kentucky University (Bowling Green, KY). Birds were housed in individual cages (34 cm x 38 cm x 46 cm) at the WKU indoor vivarium (12L:12D, lights on at 8 a.m.; 23.0° ± 1°C) and provided food (seed) and water ad libitum as well as supplemented with a single live mealworm larvae (Tenebrio molitor). The collection and study of these birds were under the approval of federal, state, and tribal entities, as well as the Institutional Animal Care and Use Committee (Animal Welfare Assurance A3558-01) at WKU. Laboratory procedures followed the National Institutes of Health’s Guide for the Use and Care of Laboratory Animals (National Research Council, 2011) and international ethical standards.

Sleep Fragmentation

To noninvasively disrupt sleep in birds, a sleep fragmentation cage was used. Developed by Cooper et al. (2019), this design employs the use of a programmable bar that moves across a cage at specified time intervals to prevent birds from sleeping. Individual birds were placed in a sleep fragmentation cage (34 cm x 38 cm x 46 cm) containing a single stationary wooden perch adjacent to food and water dishes, allowing birds access to food and water ad libitum and a place to perch. A single 16-gauge wire attached to an automated sleep fragmentation base (Lafayette Instruments, Lafayette, IN) was placed 1.0 cm above the stationary perch and moved horizontally across the width of the cage at 1-min intervals for 12 h. This rate of sleep fragmentation was used to ensure robust disruptions to sleep while still allowing birds to rest between wire movement.
single sweep of the wire lasted ca. 9 s. The flexible 16-gauge wire was used in place of a solid bar to prevent birds from perching on the moving wire. Individuals that attempted to perch on the moving wire caused it to flex and were subsequently forced to fly back to the stationary wooden perch. The cage was placed in a 28 L plastic container filled with water (ca. 4.7 cm deep) to prevent birds from resting on the bottom. Birds were then exposed to 12 h of the moving wire during either the light or dark phases (see Experimental Groups). To ensure birds subjected to the SL protocol were able to see the moving bar during the dark phase, a dim blue 8 W lightbulb that emitted ca. 1 lux was on at all times during both light and dark phases for both groups.

*Experimental Groups*

Birds were divided into two experimental groups: (1) SL (*n* = 15) where individuals were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.) when birds were typically resting or sleeping and (2) control (*n* = 14) where individuals were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) when birds were generally active. The latter group received the moving wire during 12L to account for any activity induced by the wire. Individual body mass (to the nearest 0.1 g) was recorded pre- and post-treatment.

*Blood Collection*

In December 2018, birds were administered the sleep fragmentation protocol (12 h sleep loss or control). Immediately following a 24-h treatment period (8 a.m. the following day), birds were removed from their sleep fragmentation cages and a 100 μl blood sample was taken from the alar wing vein (within 2.5 min) using a 25G hypodermic needle and collected in heparinized microhematocrit capillary tubes. Blood was
centrifuged within 5 min of collection and then plasma was extracted and stored at –20 °C until assayed.

**Corticosterone Assay**

Plasma corticosterone concentrations were determined using an ELISA kit (ADI-900-097, Enzo Life Sciences) with 96% recovery for corticosterone (0.3 ng/mL sensitivity). All reagents and standards were prepared according to the manufacturer’s instructions. Cross reactivities for the kit were <28.6% deoxycorticosterone, <2% progesterone, and <1% aldosterone. Plasma samples were diluted 1:40. The reaction was carried out in duplicate according to the kit instructions, and the average absorbance of the plate was determined using a plate reader and subtracting the absorbance at 405 nm from the absorbance at 570 nm, per the manufacturer’s instructions (BioTek Synergy H1 Hybrid Reader). Corticosterone concentrations were extrapolated from a standard curve by using a four-parameter logistic curve fit. Intra-assay variation was 2.9%.

**Executive Function Training**

Birds began executive function training in February 2019. Two types of executive function tests were used: color association and spatial learning. Subjects were tested each day at the start of the 12-h light phase (8 a.m.). Food dishes were removed 3 h prior to light cessation (5 p.m.) to ensure that birds were motivated to complete tasks for food rewards the following day. For both tests, the food rewards consisted of mealworm larvae. To test whether there was a significant effect of treatment on food motivation (satiation), we conducted a trial in which birds were given 10 min to consume 10 mealworms immediately following the final spatial learning trial (see below). For each test, trials occurred in each bird’s home cage and were recorded using two sets of cameras (iPhone...
6s) affixed to tripods. Dividers were placed between cages to prevent birds from seeing each other during trials.

Both tests were adapted from Anderson et al. (2017) and are described below. The experiment occurred in two rounds. For the first round, birds were trained to learn a color association task in which they had to meet a series of criteria to successfully complete the training (see Color Association). Once successfully trained, birds were exposed to control or SL treatment and then administered the test a final time. Birds were then given a 2-week recovery period before beginning the spatial learning task. After successful training, treatment groups were switched, birds were exposed to control or SL, and spatial memory was assessed using similar criteria (see Spatial Learning).

**Color Association**

Color association testing grids consisted of a single 6-well cell culture microplate secured to the center of a black acrylic sheet (30.5 cm x 33 cm) with mounting tape (Fig. 1a). Wells were 3.6 cm in diameter and 1.9 cm deep. One well was baited with a mealworm and covered by a blue chip while an empty well was covered by a white chip and left unrewarded. Both rewarded and unrewarded chip positions were randomly chosen each day. For this task, birds were trained to associate the blue chip with a food reward and remove the chip before moving the white chip (no food reward). In most cases, a bird would complete this task by grabbing the edge of the chip with their beak and flipping it off the well. Birds learned this task incrementally in four stages: (1) A mealworm was placed in an randomly chosen well with no chips present to train birds that a food item could be found in any well; (2) chips were positioned adjacent to two randomly chosen wells with a mealworm located in the well next to the blue chip; (3) the blue and white
chips half-covered the rewarded and unrewarded wells, respectively; and (4) the blue and white chips were completely covering the wells. The criterion for successfully completing a single trial on stages 1 and 2 was to obtain the mealworm within 10 min. The criterion for successfully completing a single trial on stages 3 and 4 was to flip over the blue chip and obtain the mealworm (within 10 min) before interacting with the white chip. Individuals had to succeed on at least three of four consecutive trials to pass stages 1–3 and four of five consecutive trials to pass stage 4. After passing stage 4, individuals began the 24-h sleep fragmentation protocol that same day to prevent birds from going 24 h without receiving the test. Birds then received the color association test a final time immediately following treatment (8 a.m. the following day). During the final trial, there was no food reward present. This was to ensure that birds would not use auditory cues to locate the mealworm and to allow for an additional quantitative parameter to be measured, termed color latency. This was a measure of the amount of time taken by an individual to first remove the blue chip, and then proceed to look elsewhere for food after realizing the food reward was missing (i.e., flipping the white chip). The criterion for successfully completing this task was to flip over the white chip after successfully passing the color association test (within 10 min).

Spatial Learning

Spatial learning testing grids consisted of the same 6-well cell culture microplates, but four wells were separated with a bandsaw and secured in each corner of the acrylic sheet using mounting tape (Fig. 1b). The back-left well was baited with a mealworm and all four wells were covered by a red chip. A red cue card was located in the back-left corner and attached to the outside of the cage to provide test subjects with a landmark for
orientation. For this task, birds were trained to remove the chip located in the back-left corner, revealing the food reward, before interacting with the chips in the other three corners. Birds learned this task in four stages: (1) A mealworm was placed in the back-left corner where the cue card was located with no chips present; (2) chips were positioned adjacent to each of the four wells; (3) chips half-covered the rewarded and unrewarded wells; and (4) chips completely covered the rewarded and unrewarded wells. The criterion for successfully completing a single trial on stages 1 and 2 was to obtain the mealworm within 10 min. The criterion for successfully completing a single trial on stages 3 and 4 was to flip over the back-left chip and obtain the mealworm (within 10 min) before interacting with any of the other three chips. Individuals had to succeed on at least three of four consecutive trials to pass stages 1–3 and four of five consecutive trials to pass stage 4. After passing stage 4, individuals were administered the 24-h sleep fragmentation protocol and then received the spatial learning test a final time immediately following treatment (8 a.m. the following day). As with color association, the mealworm was removed from the testing grid during the final trial and results were recorded remotely.

Statistical Analyses

With the exception of executive function test choice measures (see below), all data are expressed as mean ± standard error. Two-tailed unpaired t-tests were used to compare the effect of SL treatment on the following variables: (1) body mass, (2) food motivation, (3) plasma corticosterone concentrations, and (4) time spent (sec) to successfully complete the color association and spatial learning tests. A non-parametric Mann-Whitney U test was used to analyze the effect of SL treatment on color latency due to low sample size. Executive function test choice measures for color association, color latency, and spatial
learning were analyzed using Fisher’s exact tests with individual outcomes separated into two categories within 2x2 contingency tables: (1) correct choice, or (2) incorrect choice, based on the success criterion for each test measure. Failure to complete the test was categorized as an incorrect choice. All statistical tests were run in RStudio v. 1.1.456. Logarithmic transformation was necessary for some variables (plasma corticosterone concentrations, color association, spatial learning, and color latency) that initially failed the Shapiro-Wilk normality test and/or the Levene’s test of homogeneity of variance.

RESULTS

Body Mass

Percent body mass did not vary significantly between control and SL groups (two-tailed t-test, \( t = -1.73, P = 0.10 \); Fig. 2) with birds losing an average of 5.43\% (1.45 g) across groups post-treatment.

Food Motivation Test

Number of mealworms consumed did not vary significantly between the control and SL groups post-treatment (two-tailed t-test, \( t = -0.52, P = 0.61 \); Fig. 3).

Plasma Corticosterone

Concentrations of plasma corticosterone were significantly elevated in birds exposed to the SL treatment (two-tailed t-test, \( t = -2.50, P = 0.02 \); Fig. 4) compared to controls.
Executive Function Test Measures

Birds required an average of 13.82 days (± 0.36) to successfully learn the color association task. Following treatment, all SL individuals \( n = 12 \) made the correct choice whereas eight controls made the correct choice and seven made the incorrect choice. Of the birds that made the incorrect choice, two removed the white chip before the blue chip while five did not remove either chip. SL increased the accuracy of color association tests compared to controls (Fisher’s exact test, \( P = 0.008 \); Fig. 5). Among individuals that successfully completed the color association test, no significant difference in completion time was found between groups (two-tailed t-test, \( t = -0.29, P = 0.78 \); Fig 6.).

For color latency, four SL birds made the correct choice and nice made the incorrect choice while five controls made the correct choice and ten made the incorrect choice. Treatment did not significantly affect accuracy to complete the test (Fisher’s exact test, \( P = 0.99 \); Fig. 7). For individuals that proceeded to remove the white chip after successfully completing the color association test, no significant difference in completion time was found between groups (two sample t-test, \( t = 0.36, P = 0.73 \); Fig. 8).

Birds required an average of 13.52 days (± 0.23) to successfully learn the spatial task. For the SL group \( n = 8 \), four birds made the correct choice and four made the incorrect choice. Two control individuals made the correct choice and four made the incorrect choice. Of the birds that made the incorrect choice, six removed the wrong chip first (i.e., front right, front left, or back right) before moving the back left chip while one did not remove any chips. Treatment had no significant effect upon test accuracy (Fisher’s exact test, \( P = 0.63 \); Fig. 9). Unfortunately, sample size was too low for statistical analysis of spatial learning completion time between groups (Fig. 10).
DISCUSSION

The findings of this study provide evidence that when subjected to acute sleep loss (i.e., 12 h of wire movement during the dark phase), the arctic-breeding Lapland longspur demonstrates behavioral, but not physiological, resilience. More specifically, the sleep loss protocol triggered an adrenocortical response indicated by significantly elevated baseline corticosterone concentrations while showing no evidence of decrements to executive function when presented with a nonspatial executive function task. Therefore, the hypothesis that longspurs demonstrate behavioral resilience in response to a series of executive function tests is supported. However, longspurs are still responsive physiologically to sleep loss.

To date, most studies documenting increased glucocorticoid levels from sleep restriction in birds have used human intervention to awaken birds every 3–5 min (Rattenborg et al., 2004; Jones et al., 2008, 2010), albeit without handling them. The use of the sweeping bar method to induce sleep loss eliminated the need for human interaction and was effective from an adrenocortical response standpoint. Birds subjected to the moving wire during the dark phase (every 1 min for 12 h total) exhibited significantly elevated plasma corticosterone concentrations relative to controls. These results are consistent with previous studies investigating the effect of acute sleep fragmentation on corticosterone concentrations in mice and birds using the sweeping bar method (Dumaine and Ashley, 2015; Cooper et al., 2019). In addition, percent change in body mass did not vary significantly between control and treatment group. Though studies have shown that food restriction and changes in body mass affect circulating plasma corticosterone levels in mice and birds (Sockman and Schwabl, 2001; Singleton and Garland, 2019; Robart et
al., 2019), these data taken together indicate that the administration of the SL protocol concomitant with 3 h food restriction did not differentially affect body mass across groups. In addition, this treatment did not affect satiety levels in birds. SL birds exhibited similar satiety levels compared with controls, as measured by the number of mealworms consumed after the sleep fragmentation protocol. Therefore, differences in performance in executive function observed cannot be attributed to differences in hunger. Although the sweeping bar method was effective in inducing wakefulness during the dark phase, it is unclear whether birds were experiencing fragmented sleep or complete sleep deprivation. Therefore, this method will need to be refined to allow for assessment of electroencephalographic and electromyographic biopotentials to assess the degree of sleep disruption.

Despite the apparent reduction in sleep in some migratory birds during migration and breeding (Fuchs et al., 2006; Jones et al., 2010; Lesku et al., 2012; Ashley et al., 2013), observations of songbirds in the wild suggest a capacity for maintaining high levels of cognitive and physical function, including migratory navigation, territory establishment and defense, foraging, and predator evasion. Moreover, mounting evidence suggests that secretion of glucocorticoids in the event of a stressful situation (e.g., sleep restriction) plays a critical role in memory consolidation in humans, rats, and some birds (Sandi and Rose, 1994, 1997; Liu et al., 1999; Roozendaal et al., 1999; Buchanan and Lovallo, 2001).

SL was significantly associated with an individual’s increased likelihood of correctly completing the color association test, a finding that could be tied to elevated corticosterone levels. However, SL did not affect the amount of time taken to successfully complete the test. The results of the color latency test, used as a proxy for behavioral
flexibility after a mild stressor (i.e., acute sleep loss), indicated that 12 h of sleep loss did not significantly alter the amount of time taken to remove the white chip after first completing the color association test nor did it affect the amount of time taken to successfully complete the color latency test. Taken together with similar findings of behavioral resilience to sleep loss in the migratory white-crowned sparrow using an operant task (Rattenborg et al., 2004; Jones et al., 2010), my results from the color association and color latency tests administered to Lapland longspurs suggest that these particular arctic-breeding songbirds are also resilient to deficits in nonspatial memory after a round of sleep fragmentation (i.e., bar movement at night for 12 h) in the laboratory.

For the spatial learning test, treatment was not significantly associated with an individual’s likelihood of making the correct test choice nor did treatment alter the amount of time taken to successfully complete the test. It is well known that the hippocampal region of the brain is particularly sensitive to stress (see Lupien and Lepage, 2001 for review). Additionally, accumulating evidence suggests that consolidation and retrieval of spatial memory is more heavily dependent upon the integrity of the hippocampus, an area of the brain known to exhibit more profound deleterious effects after sleep loss, relative to non-spatial memory (Guan et al., 2004; Broadbent et al., 2004) in both mammals (McDonald et al., 1993) and birds (Krebs et al., 1996; Clayton, 1995). Interestingly, while longspurs learned the color association and spatial learning tasks in a similar amount of time (ca. 13.5 days per task), dramatically reduced response rates (nearly 50% failure) on the spatial learning test after SL protocol regardless of treatment were observed. The SL protocol included transferring birds from their home cage, where they received the executive function training, into a sleep fragmentation chamber for 24 h, and then placed
into a third room where they were administered the executive function tests a final time. Therefore, it is possible that the change in environment (i.e., the testing room) led to additional stress that caused individuals to fail or not participate in the executive function task regardless of treatment.

While the results obtained from the color association task align with current literature, the results from the spatial learning task appear to be more enigmatic. It seems paradoxical that greater detriments would be observed in spatial memory compared to nonspatial memory in these birds. During their breeding season, male Lapland longspurs would presumably need to be more resilient to the detriments of sleep loss on spatial memory than nonspatial memory to navigate the tundra landscape with limited markers for spatial orientation. However, it is possible that these birds use other nonspatial cues to successfully locate territories and nest sites.

Future studies should assess whether temperate-breeding birds that breed in regions precluding 24 h of sunlight, such as the chestnut-collared longspur (*Calcarius ornatus*), are more sensitive to the behavioral and physiological effects of sleep loss relative to polar-breeding birds. Lastly, experimentally manipulating corticosterone levels and assessing executive function in these birds could elucidate the influence that stress hormones have upon behavioral adaptations in high-latitude environments.
Fig. 1. Executive function test cage designs

Photographs of the cage designs used for the executive function tests (cage tops removed). a, A cage set up for the color association test. A 6-well cell culture microplate is secured to the center of an acrylic insert (30.5 cm x 33.0 cm). A randomly chosen well covered by the blue chip is consistently baited with a mealworm while the randomly chosen well covered by the white chip remains unrewarded. b, A cage set up for the spatial learning test. Single wells were secured in each of the four corners of the cage on top of an acrylic insert. The well in the back-left corner is consistently baited with a mealworm. A red cue card (5.7 cm x 8.9 cm) is affixed to the outside of the cage directly behind the rewarded well (yellow arrow) to provide test subjects with a landmark for orientation.
Fig. 2. Percentage change in body mass

Percentage change in body mass (mean ± SE, n = 14 per group) of Lapland longspurs immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the experimental sleep loss (SL) group were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). Percentage change in body mass was not significantly different between groups (two sample t-test, t = -1.73, P = 0.10). P < 0.05 is considered statistically significant.
Fig. 3. Post-treatment food motivation (satiety) test

The number of mealworms consumed (mean ± SE, n = 8 per group) by Lapland longspurs immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the experimental sleep loss (SL) birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). Number of mealworms consumed was not significantly different between groups (two sample t-test, $t = -0.52, P = 0.61$). $P < 0.05$ is considered statistically significant.
Fig. 4. Baseline plasma corticosterone levels

Plasma corticosterone levels (ng/mL; mean ± SE) in Lapland longspurs sampled immediately following 24 h of sleep fragmentation protocol. Controls (n = 13) were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the experimental sleep loss (SL) group (n = 15) was exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). An asterisk indicates a significant effect of the SL treatment (P = 0.02). P < 0.05 is considered statistically significant.
Fig. 5. Color association test scores

Individual color association test scores for Lapland longspurs in the control ($n = 15$) and experimental sleep loss (SL) ($n = 12$) groups. The color association test was administered with scores recorded immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). An asterisk indicates a significant association between treatment and test choice (Fisher’s exact test, $P = 0.008$). $P < 0.05$ is considered statistically significant.
Fig. 6. Color association test completion time

Log-transformed color association completion time for Lapland longspurs in the control ($n = 8$) and experimental sleep loss (SL) ($n = 11$) groups. The color association test was administered immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). Time taken to successfully complete the color association test was not significantly different between groups (two sample t-test, $t = -0.29$, $P = 0.78$). $P < 0.05$ is considered statistically significant.
Fig. 7. Color latency test scores

Individual color latency test scores for Lapland longspurs in the control \((n = 15)\) and experimental sleep loss (SL) \((n = 13)\) groups. Color latency measures were recorded in tandem with the color association test, administered immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). No significant association between treatment and test choice was found (Fisher’s exact test, \(P = 0.99\)). \(P < 0.05\) is considered statistically significant.
Fig. 8. Color latency test completion time

Log-transformed color latency completion time for Lapland longspurs in the control ($n = 5$) and experimental sleep loss (SL) ($n = 4$) groups. Color latency measures were recorded in tandem with the color association test, administered immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). Time taken to successfully complete the color latency test was not significantly different between groups (Mann–Whitney $U$ test, $U = 14$, $P = 0.41$). $P < 0.05$ is considered statistically significant.
Individual spatial learning test scores for Lapland longspurs in the control \((n = 6)\) and experimental sleep loss (SL) \((n = 8)\) groups. The color association test was administered with scores recorded immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). No significant association between treatment test choice was found (Fisher’s exact test, \(P = 0.63\)). \(P < 0.05\) is considered statistically significant.
Fig. 10. Spatial learning test completion time

Log-transformed spatial learning completion time for Lapland longspurs in the control ($n = 2$) and experimental sleep loss (SL) ($n = 4$) groups. The spatial learning test was administered immediately following 24 h of sleep fragmentation protocol. Controls were exposed to the moving wire during the light phase (8 a.m. to 8 p.m.) while the SL birds were exposed to the moving wire during the dark phase (8 p.m. to 8 a.m.). Sample size was too low for a statistical inference.
REFERENCES


