Effects of Pharmacologically-Induced Sleep Loss on Parental Care in Arctic-Breeding Songbirds

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EFFECTS OF PHARMACOLOGICALLY-INDUCED SLEEP LOSS ON PARENTAL
CARE IN ARCTIC-BREEDING SONGBIRDS

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Master of Science

By
Wesley Ivar Payette

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EFFECTS OF PHARMACOLOGICALLY-INDUCED SLEEP LOSS ON PARENTAL CARE IN ARCTIC-BREEDING SONGBIRDS

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This thesis is dedicated to Aaron Sullivan, Ph.D.

ACKNOWLEDGEMENTS

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EFFECTS OF PHARMACOLOGICALLY-INDUCED SLEEP LOSS ON PARENTAL
CARE IN ARCTIC-BREEDING SONGBIRDS

Wesley Payette   May 2020   35 Pages

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Sleep loss is well known to impair cognitive function, immunological responses, and general well-being in humans. However, sleep requirements in mammals and birds may vary dramatically, especially with changes in environment. In circumpolar regions with continuous light, sleep requirements may be little, particularly in breeding birds. The effects of sleep loss on several fitness parameters were examined in two species of Arctic-breeding passerine birds: Lapland longspurs (*Calcarius lapponicus*) and snow buntings (*Plectrophenax nivalis*). Adult males were implanted during the nestling phase (4 days post-hatch) with osmotic pumps containing an anti-narcolepsy drug, modafinil, to extend the active period for 72 h. Nestlings were weighed on day 2 and day 7 following hatching. In addition, 1-h observations of nestling feeding rates on day 6 post-hatch were conducted. Male longspurs receiving modafinil were less likely to feed nestlings and spend time at the nest but spent more time around the nest than controls. I observed no change in growth rates for longspur nests, but treatment nests tended to fledge a day later. Modafinil had no visible impact on male or female snow bunting behavior; growth rates and time to fledge were similar between groups. I suggest male longspurs require more energy to maintain vigilance at their nests because they build nests in open tundra, where predation is more likely. As snow buntings are functionally cavity nesters, their nests may not require the same levels of vigilance, allowing time for males to rest following provisioning.

INTRODUCTION

The purpose of sleep is not fully understood but the benefits of a good night's rest are well established (Rolls, 2011). Sleep loss is usually associated with an overall lack of responsiveness, reduced motor activity, and decreased metabolism (Seigel, 2009; Lesku and Rattenborg, 2014). Lack of sleep in humans has been shown to have serious cognitive consequences (Goel et al., 2009). Furthermore, it has been linked to several health issues including diabetes (Cappuccio et al., 2009), obesity (Cappuccio et al., 2008), hypertension (Fung et al., 2011), and cardiovascular diseases (Miller and Cappuccio, 2007). Clearly, a lack of sleep is detrimental to overall well-being.

However, the amount of sleep per night required by many species is quite variable (Campbell and Tobler, 1984; Lima et al., 2005; Siegel, 2009). While most animals studied to date require sleep or sleep-like behavior, certain mammals may sleep for only a few hours per night (giraffes, elephants), while some bat species require more than twenty hours (Siegel, 2009). Many species of birds also differ in their sleep requirements, which may change during breeding and migration (Ball and Amlaner, 1983; Rattenborg et al., 2004; Lima et al., 2005; Fuchs et al., 2006; Steiger et al., 2013). While on the wing for several days at a time, great frigatebirds (*Fregata minor*) can sleep unihemispherically, spending only a fraction of the time sleeping in air as they might on land (Rattenborg et al., 2016). Additionally, non-breeding common swifts (*Apus apus*) may spend almost 10 consecutive months airborne, with less than 1% of their time spent not flying, suggesting they sleep on the wing (Hedenström et al., 2016). As the amount of sleep required varies between species, the way sleep is obtained also depends on the seasonal and environmental contexts of the organism.

Trade-offs may occur as individuals lose sleep while on alert to the presence of predators or conspecifics, especially during the breeding season. Reproductive effort can challenge survivorship (Montgomerie and Weatherhead, 1988; Stearns, 1989; Santos and Nakagawa, 2012) and maintaining vigilance behavior, adequate feeding rates, and brooding time may compromise sleep. In pink-footed geese (*Anser brachyrhynchus*), vigilance in parents is maintained through head-up posture, proximity to other geese, and active defense of the brood at the expense of sleeping and reduced self-feeding time (John and Inglis, 1978). These behaviors are also apparent in tundra swans (*Cygnus columbianu*) (Earsnt, 2002). Additionally, brooding female great tits (*Parus major*) infected with an ectoparasite allocate less time to sleeping than uninfected parents but maintain provisioning rates despite setting aside time for anti-parasite behavior (Christe et al., 1996). In some species of grassland birds, parents undergo interrupted sleep during the night to stay vigilant, suggesting the benefits of nocturnal vigilance behavior outweigh the cost of poor sleep (Slay et al., 2012). Clearly, sleep can be compromised while maintaining nestling health. However, in species that regularly undergo seasonal sleep deprivation, it is unknown just how much sleep is required to maintain nestling care.

At higher latitudes, extreme photoperiodicity surrounding the solstices create seasons of constant day or constant night. For bird species that breed north of the Arctic Circle in summer, these conditions can allow for adaptive sleep rhythms that many use to their advantage. Pectoral sandpipers (*Calidris melanotos*) contrast long periods of activity with very short periods of sleep (Lesku et al., 2012). This lack of sleep seems to offer a competitive reproductive advantage, as the most active males tend to produce the most

offspring. In addition, semipalmated sandpipers (*Calidris pusilla*) utilize several

circadian rhythms during the breeding season, which may also function to increase

mating opportunities (Steiger et al., 2013). Furthermore, Lapland longspurs (*Calcarius

lapponicus;* LALOs) are mostly inactive from approximately midnight to 0400 h during

the breeding season but will maintain territorial aggression throughout the 24 h period

(Ashley et al., 2013; Steiger et al., 2013; Ashley et al., 2014). While it is clear some

species in arctic regions experience seasonal changes in sleep patterns, little work has

investigated how parental care may be influenced by manipulation of these patterns.

During summer in the high arctic, sleep requirements in animals may be different

due to a short breeding season and constant daylight. I am interested in the extent to

which sleep requirements in Arctic-breeding songbirds affect their ability to provide

adequate parental care. The effects of pharmacologically modified sleep loss in birds is

understudied and may be especially interesting in Arctic species. Here, I investigated the

effects of an anti-narcolepsy stimulant (modafinil) on two free-living songbird species:

the Lapland longspur and the snow bunting (*Plectrophenax nivalis*; SNBU). Both species

breed in circumpolar regions and experience a 24 h daylight regime during the summer.

SNBUs tend to nest in cracks in tussocks, rocky hillsides, and man-made structures, such

as nest boxes, eaves, or any cavity that offers protection (Montgomerie and Lyon, 2011).

LALOs nest almost exclusively in open tundra (Hussell and Montgomerie, 2002) and are

naturally more prone to nest predation from predators such as jaegers, gulls, arctic fox,

and ermine (Liebezeit and Zack, 2008). Since SNBU nests are better protected and more

cryptic, the nest defense strategies of the species may be different from LALOs, despite

their sympatry (Montgomerie and Weatherhead, 1988). Additionally, SNBUs may be

more likely to exhibit promiscuous behavior than longspurs, which may also affect sleep patterns (Hoset et al., 2014). Differences in the life-history traits between the two species may lead to a difference in the amount of sleep required for reproductive success and whether sleep loss will negatively affect reproductive behavior.

The major aim of this study was to pharmacologically expand the active period of free-living LALOs and SNBUs using short-term (72-h) modafinil exposure through osmotic pump infusion. I hypothesized that males receiving the stimulant would initially increase parental care, as they would be spending more time active throughout the day. I therefore expected nestling growth rates to increase for males receiving modafinil compared with controls during this period. Additionally, after the stimulant wore off, I expected to see a reduction in nestling health and survival as males caught up on sleep lost during the active period.

METHODS

Captive Experiments and Study Species

All experiments described below were approved by the Institutional Animal Care and Use Committee (IACUC) at Western Kentucky University, and appropriate state, federal, and tribal entities for field studies in northern Alaska (see below).

Two drugs were tested to examine their efficacy in extending the active ("awake") period of bird species. To assess the activity profiles of birds housed in individual cages (55 cm x 25 cm x 25 cm), I used the Limelight video tracking program (Actimetrics) to measure a bird's activity over a set time period. The top of the cage was removed, and a

piece of plexiglass fit onto the top so the camera could observe the bird without

obtrusion. Birds were fed *ad libitum.* Our first trial involved caffeine. I attempted to use

0.1 ml injections of 10, 20 and 30 mg/ml of caffeine dissolved in DI water (vehicle) by

vortexing to extend the active period of captive adult zebra finches (*Taeniopygia guttata)*,

using each bird as its own control. Birds were tested as controls first and given a 48-h rest

period between trials. I tested two birds at a time with lights on between 0800 h and 2000

h for periods of 8, 10 and 12 h. The site of injection was prepared with a cotton ball

soaked in 70% ethanol to expose the skin of the abdomen and allow for subcutaneous

injections. However, caffeine appeared to have no effect compared to controls (data not

shown). In one trial involving 30mg/ml, the injection was lethal in the first hour. I then

attempted to use modafinil, an anti-narcolepsy drug for humans. Again, captive adult

zebra finches were used to determine the appropriate dose that increased the range of

activity (Figure 1). Birds were placed in individual cages in a room with constant light

(24L:0D) to mimic light conditions during the arctic summer. I found that 75 mg/kg BW

modafinil increased the active period during the subjective night (lights still on) when

compared to controls. I then tested the effect of modafinil using an osmotic pump

delivery method that would eventually be used in the field that could putatively induce

effects over a period of 72 h. For these preliminary studies, our lab had several captive

Lapland longspurs that were captured previously near Utqiagvik, Alaska in 2018 and

brought to the Western Kentucky University vivarium for another study. An osmotic

pump (Azlet 1003D) was filled with 75 mg/kg BW modafinil dissolved in a 50%

DMSO/50% polyethylene glycol solution. This particular vehicle was used because the

larger concentrations of modafinil were not very soluble in other types of vehicle (e.g.,

water, 100% DMSO, etc). Pumps contained 100 ul of solution and released solution at a rate of 1 ul/hr. For implantation, the surgical site was first prepped by using a cotton ball soaked with 70% ethanol to expose the skin directly underneath the neck and the scapular feathers. Then, a small incision (approx. 1 cm) in the skin between the bird's shoulder blades was made using fine-scale micro-dissecting scissors (Roboz Surgical Instrument Co.) that was just wide enough to accommodate sliding the implant underneath the skin. The incision was sealed with veterinarian surgical glue. I observed an increase in the range of activity in the bird receiving the pump containing 75 mg/kg BW modafinil dissolved in 50% dimethyl sulfoxide (DMSO) and 50% polyethylene glycol for each of the three days the pump was active (Figure 2). Again, the photoperiod in this experiment was 24L:0D. This dose and delivery method were used in the field trials below.

Field Site and Study Species

Free-living Lapland longspurs and snow buntings were studied in the general vicinity of Utqiagvik (formerly known as Barrow), Alaska (71°N, 156°W) during the summer of 2019 (June 2 to July 14). As longspurs nest in tundra, most of our field sites for this species were located in coastal tundra within walkable distance of the Barrow Arctic Research Station or Cakeeater/Gaswell Road (the longest drivable road in Utqiagvik). The coastal tundra consists of polygons, sedge meadows, drained lake basins, and wetlands, and is poorly drained with little topographical relief. The most common plant species are *Carex aquatilis* (a sedge), *Eriophorum* (cottongrass), and *Dupontia fischeri* (Fisher's tundragrass), as well as various mosses which are prevalent in low, wet coastal sites.

The majority of snow buntings were studied at the National Arctic Research Laboratory/Ilisagvik campus, where artificial nest boxes have been erected to monitor this particular population for several decades. In addition, these birds also nest in other man-made structures on the campus, such as vehicles, machinery, and other abandoned structures. However, in 2019, there were a number of SNBUs that nested in the costal tundra either in polygon tundra frost heaves, or underneath artificial debris on the tundra (e.g., discarded building supplies, such as plywood).

Nest Monitoring

During the month of June, our research team actively searched for nests of both species and monitored them until hatch. Thereafter, we monitored these nests daily to ascertain date of hatch (defined as 1 or more chicks hatched from a clutch, termed Day 0). On Day 2 post-hatch, nestlings were weighed with a small, battery-operated portable digital scale and their toenails were marked with colored nail polish for individual identification. Handling time was limited to keep the chicks as warm as possible, and nestlings were kept inside a heated cooler to protect them from exposure. Day 4 involved capturing and implanting the resident male with an osmotic pump filled with modafinil or vehicle (see below in *Osmotic Pump Implantation*). On Day 6, we assessed parental behavior of focal male and female birds (see below in *Parental Behavior Observations)*. On Day 7, we weighed, banded and obtained a blood sample from each nestling. Afterwards, the nest was monitored for fledging or depredation. We also attempted to recapture and explant the male if possible.

Osmotic Pump Implantation

On Day 4, we attempted to capture resident adult males at each nest and implanted them with an Azlet 1003D osmotic pump containing modafinil (75 mg/kg for each day the pump was active, or vehicle (1:1 ratio of polyethylene glycol and DMSO). Males were captured using potter traps or claptraps baited with seed accompanied by a live decoy with acoustic playback from a small speaker. Once captured, males were measured for tarsus length, wing length, and body mass (to the nearest 0.25 g) for each captured bird. Fat score was ascertained by estimating the extent of furcular and abdominal fat deposition using a semiquantitative scale (0-5 with 5 having the highest fat stores) as developed by Wingfield and Farner (1978). These two scores were then averaged for each bird. Osmotic pumps were implanted underneath the skin of birds as described above in *Captive Experiments & Study Species*. Subjects were also banded with a unique combination of plastic colored leg bands for individual identification, as well as an aluminum leg band (United States Geological Service). Birds were then released on an adjacent territory after being warmed in a bird bag for 5-10 minutes, as we attempted to minimize the stress of capturing and handling with release at the bird's own territory. Following fledging, we attempted to recapture males to remove explants and assess body condition. Due to the number of days between implant and explant, we were unable to assess the immediate effects of modafinil. Instead, we used fat score and mass to assess body condition after the effects of the drug wore off.

Parental Behavior Observations

On Day 6 of each nest where a male was implanted, we conducted 60 min. behavioral observations of the focal male and breeding female within 20 m of the nest. The observations occurred from 0900 to 1500 AKDT. The observer was > 20 m away

from the nest and was naïve to the treatment of the focal male. The following behavioral

parameters were measured: (1) duration spent within 5 m of the nest (s), (2) number of

visits to nest, (3) time spent on nest (s), and (4) number of flights of male (which includes

chasing another male). In some cases, birds left the nest area and were considered "out of

view" and thus greater than 5 m from the nest. Behavioral observations were recorded in

a digital dictaphone.

Finally, we measured nestling growth rates for both species by comparing the

difference between means for groups using percent increase in grams per day and percent

increase in the smallest and the largest chick at day 7 in each brood. We also compared

the percent increase in grams per day for each chick to the next largest chick, from

smallest to largest.

Statistical Analyses

Statistical analyses were done in RStudio using the function wilcox.test to

perform non-parametric Mann-Whitney U tests on groups of data, since most of the data

was non-normal even after transformation. Tests for parametric data were done using the

t.test function.

RESULTS

Field Study

Over the course of the field season we found and monitored 59 longspur nests and

46 snow bunting nests. By Day 6 post-hatch, 67% LALO of nests had failed whereas

15% of SNBU nests failed. Nineteen ($n=8$ modafinil; $n=11$ vehicle) longspur nests

survived until Day 6 and could be used for behavioral observations, and 16 ($n=8$

modafinil; n=8 vehicle) survived until Day 7 and were used to measure growth rates. Additionally, 24 SNBU (*n=10* modafinil, *n=14* vehicle) nests were monitored for parental behavior and nestling growth rates. Clutch size averaged 5.2 ±0.36 nestlings for LALOs and 5.8 ±0.25 for SNBUs. Mean days to fledge was 11 ±0.32 in LALOs and 15 ±0.33 in SNBUs.

Parental Behavior

LALO males receiving modafinil made fewer visits to the nest than controls (Fig. 3; *U*=19; (tied) *p*=0.032). Males receiving the drug tended to spend less time on the nest than controls (Fig. 4; *U*= 21; (tied) *p*=0.049) and tended to spend more time within 5 m of the nest than controls (Fig. 5; *U*= 22; (tied) *p*=0.069). However, modafinil treatment in LALO males did not modify female behavior. We found no difference between groups for female time spent on the nest (Fig. 6; *U*=4; (tied) *p*=0.87.) or the number of female visits to the nest (Fig. 7; U=38; (tied) *p*=0.62). We recaptured and explanted three males following implantation but were unable to compare means between groups because of our small sample size.

Male SNBUs receiving modafinil did not exhibit any differences in time spent on the nest (Fig 8; *df*=22; *t*=-0.24; *p*=0.81) or visits to the nest (Fig. 9; *df*= 21; t=-0.25; *p*=0.81) compared with males receiving vehicle. Additionally, modafinil treatment in males did not significantly influence parental behavior in females, as measured by the number of nest visits (Fig. 10; *df*=8.9; *t*=0.75; *p*=0.47) or time spent at the nest (Fig 11; *df*=22; *t*=-1.5; *p*=0.14). We recaptured and explanted nine males following fledging. One male had no implant and we assumed it explanted itself in the thirteen days since

implantation. There was no difference in cumulative fat score (*df*=7; *t*=1.38; *p*=0.47) or mass (*df*=7; *t*=0.550; *p*=0.599) between groups following explanting.

Nestling Growth Rates

We did not observe any difference between means in any of the growth rate comparisons for either species (Fig. 12, 13; Table 1). However, we did observe a difference in time to fledge between groups for LALOs (Fig. 14; *df*=12; *t*=2.35; *p*=0.037) but not for SNBUs (Fig. 15; *df*=20; *t*=0.38; *p*=0.71). Nests of LALOs where males received modafinil fledged about one day later than controls. The overall combined average growth rate for SNBUs was 3.65 g/d and 2.93g/d for LALOs.

DISCUSSION

Behavioral observations suggest pharmacologically expanding the activity profiles of LALOs modified their parental behavior. LALO males receiving modafinil exhibited fewer visits to the nest, less time spent on the nest, and more time spent within 5 m of the nest compared to controls. Interestingly, I saw no evidence that SNBU males receiving modafinil modified their behavior when compared to controls. Additionally, female behavior did not change as a result of male implantation in either species. Finally, no change in growth rates were observed between groups in either species despite an obvious lack of parental care in male LALOs. However, nestlings of LALOs receiving modafinil tended to fledge a day later than controls.

In LALOs, modification of behavior was apparent, suggesting sleep loss does affect parental care. I suggest modafinil extended the activity profile of free-living male

LALOs on day four and five post-hatch to the extent that parental care was compromised on day six because of sleep loss. Males receiving the drug spent more time around the nest, but less time going to the nest, apparently assuming vigilance behavior rather than feeding behavior. Vigilance may be less costly than feeding, as males typically stay within a few meters of the nest, calling as they make their way around the perimeter, accompanied by short flights. Feeding typically consists of longer flights away from the nest, foraging, and a return to feed the nestlings. Males that fed did so anywhere from one to fourteen times per hour. Rates like this may be too physically demanding for sleep-deprived individuals to accomplish.

Vigilance and watchfulness should be adaptive because sleeping unawares increases the threat of predation (Rattenborg et al., 1999; Lima et al., 2005; Ferretti et al., 2019). LALOs that sleep at the expense of vigilance and provisioning should be selected against because they will be less effective at taking care of their broods. LALOs undergoing sleep loss may need to choose between sleeping and provisioning, as the latter may demand more of the former because of its energy cost. I suggest a compromise: sleep deprived LALOs may switch to vigilance, a less expensive but still protective behavior that maintains nest watchfulness but may not incur the same costs in sleep. In this scenario, an active guard is maintained at the nest, increasing the chances of total nest success, despite a possible delay in nestling growth and fledge rates.

While clear behavioral changes were observed in LALOs, SNBUs did not exhibit this same change. Behavioral means between modafinil and vehicle groups were similar overall. I suggest the difference in nesting habits may be partially responsible. While LALOs create cryptic nests in exposed open tundra, SNBUs lay exclusively in nest

boxes, holes, or man-made objects. Regardless of the difficulty a predator may have finding nests of either species, predation of a SNBU nest is more difficult. This is partially supported by a 15% nest failure rate in SNBUs compared to 67% in LALOs during our study season. As a result, we believe SNBUs do not require the same level of vigilance when protecting their nests and may have more time for resting. Anecdotal observations of SNBU and LALO nests between 0000 h and 0100 h support this. Part of the reason I did not include a metric for SNBU time within 5 m of the nest is just so: SNBU males do not maintain active, consistent guard around their nest during the day or night, and during behavioral observations spent time near the nest only to feed. In LALOs, I frequently observed the male spending time around the nest before or after feeding, typically exhibiting vigilance behavior.

As seen in other studies, (John and Inglis, 1978; Christie et al., 1996; Earsnt, 2002; Slay et al., 2012) increased parental care at the expense of sleep may be an overall fitness advantage, despite the more immediate health trade-offs. However, I am still uncertain as to why LALO nestling growth was maintained through day seven despite an obvious change in male parental care on day six. I initially hypothesized males receiving modafinil would increase feeding behavior. If this had been the case on day four or five, we would expect an increase in growth rates by day seven compared to controls. In the case males were unable to provide adequate care, I would expect females to increase care relative to controls. This was not the case. I am uncertain as to why parental care in males decreased but nestling growth and female parental care remained unchanged through day seven. It is possible females were unable to compensate for a lack of male support. Whittingham et al. (1994) report higher rates of nest provisioning in widowed female tree

swallows (*Tachycineta bicolor*) but suggest that feeding rates may not be the best indicator of female care. Rather, nestling success and quality may be more relevant metrics in determining female compensation.

While no change in nestling growth rate was observed between groups, LALO nests of males receiving modafinil tended to fledge one day later than controls. I speculate that a reduction in nestling growth occurred after day seven that we were unable to measure because after this period, there is a greater risk of premature force-fledging that can occur. Even though feeding rates in males receiving modafinil were lower than controls on day six, the effects of this behavior may not have been apparent until after day seven and were manifested in fledging dates. As nest predation rates can be high, fast growth rates and earlier fledging may increase recruitment (Verboven and Visser, 1998; Maness and Anderson, 2013). The later dates to fledge may have been indicative of lower growth rates, which was probably a result of the decrease in male feeding rates. This may be considered a slight, but important fitness cost in nests of males receiving modafinil. If the female is unable to compensate for the sleep-deprived male, then nestlings may not reach proper fledging mass as early and may be more vulnerable to predation the longer they remain in the nest.

Furthermore, these findings suggest that longspurs, a ground-nesting species, may be more susceptible to pharmacological sleep loss than SNBUs, a cavity-nester from a fitness perspective. I propose that selection should favor a sleep schedule in longspurs that is less flexible than SNBUs. Previous data from our laboratory (Ashley et al., 2013) and others (Steiger et al., 2013) have shown that male LALOs have a consistent diel rhythm in activity profiles with a quiescence period that occurs from *ca.* 00:00 to 4:00 am

AKDT (Ashley et al., 2013) In addition, preliminary data from our lab suggests that the majority of sleep bouts occurs during this inactive period (N.T. Ashley, unpublished data). Although a similar activity profile has been observed in SNBUs (N. T. Ashley, pers. obs.), we propose that there is greater flexibility of male SNBUs in their sleep requirements partially due to their protected nest sites that require little to no vigilance.

In summary, I initially hypothesized that males receiving modafinil would temporarily increase parental care and thus induce an increase in nestling growth rates. I then expected a decline in nestling growth rate as males caught up on sleep lost in the extended active period. I observed partially the opposite. Male LALOs receiving modafinil changed their behavior from a time-consuming, expensive feeding regime, to a less active but still potentially useful vigilance regime. Additionally, nests where males received modafinil tended to fledge a day later, although no changes in growth rates were observed. Interestingly, SNBUs did not exhibit any changes from modafinil treatment, which may be in part due to their well-protected nests. While this project sheds light on what is tolerable under conditions of sleep loss in Arctic-breeding songbirds, more work must be done to examine the interspecific differences in sleep demand and tolerance in arctic-adapted species.
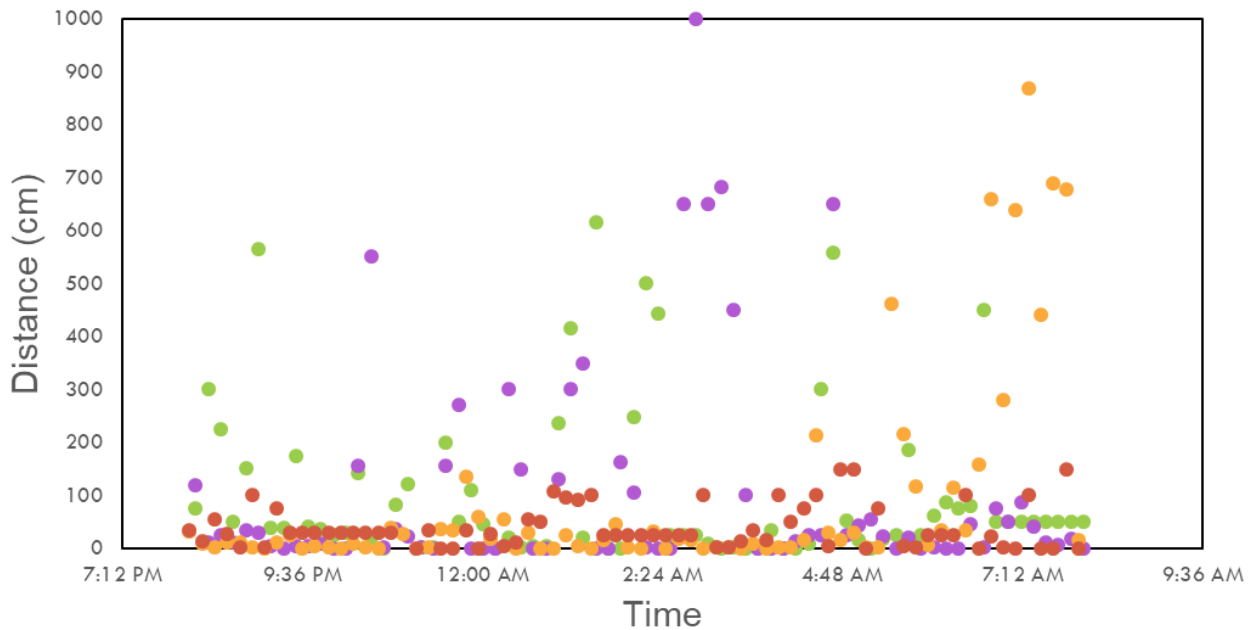
Fig. 1. Captive zebra finch modafinil trials

Four zebra finches were placed in a room with constant light to mimic polar-day conditions (24L:0D) and injected subcutaneously with 75 mg/kg modafinil or vehicle. Each point represents the cumulative distance moving in individual cages over ten-minute periods. Controls are shown in orange (0.15 ml) and red (0.20 ml). Birds that received the drug are shown in purple (0.20 ml) and green (0.15 ml). There are noticeable peaks during the subjective night for birds receiving the drug when compared to controls.
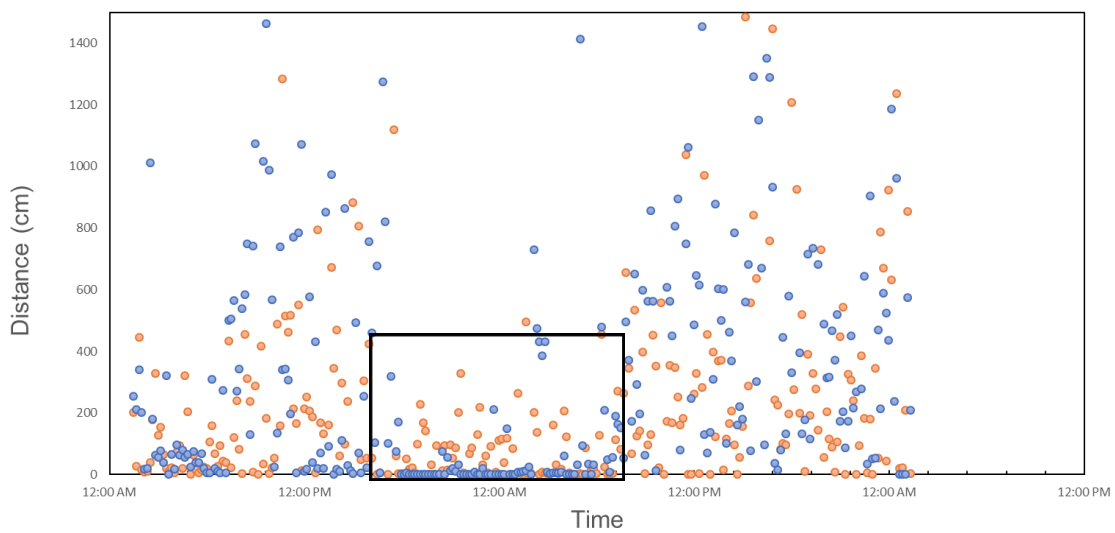
Fig. 2. Captive LALO modafinil trials

Activity profile of two captive LALOs during a 48-h validation trial
using osmotic pumps to deliver solution. Points are distances travelled
every ten minutes in individual cages. Birds were exposed to a constant
light photoperiod (24L:0D). Orange is modafinil, blue is vehicle.
Overall, activity during subjective nighttime appears greater for the
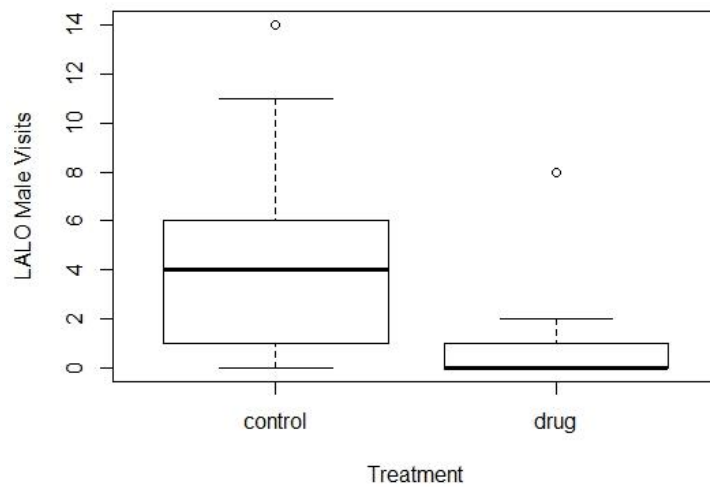individual receiving modafinil as indicated by the black box.

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Fig. 3. Male LALO nest visits

The total number of visits to the nest for male LALOs during a 1 h observation period on day six post-hatch. Males receiving modafinil (*n*=8) were less likely to visit the nest compared to controls (*n*=11).
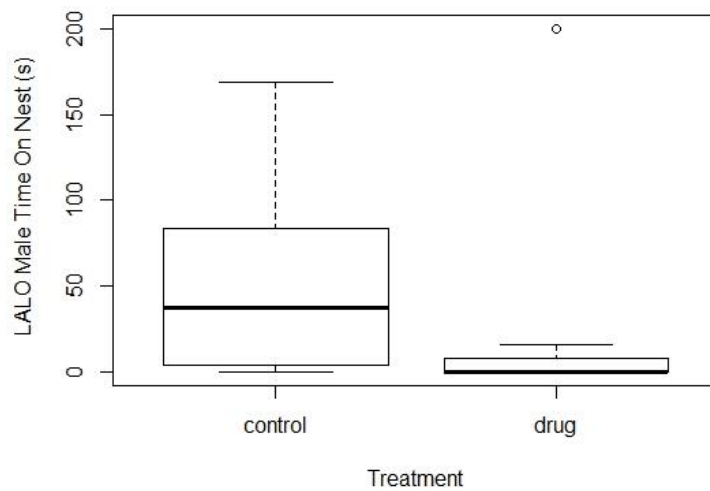
Fig. 4. Male LALO time on nest

The total amount of time spent on the nest for male LALOs during a
1 h observation period on day six post-hatch. Individuals receiving
modafinil ($n=8$) were significantly less likely to spend time on the
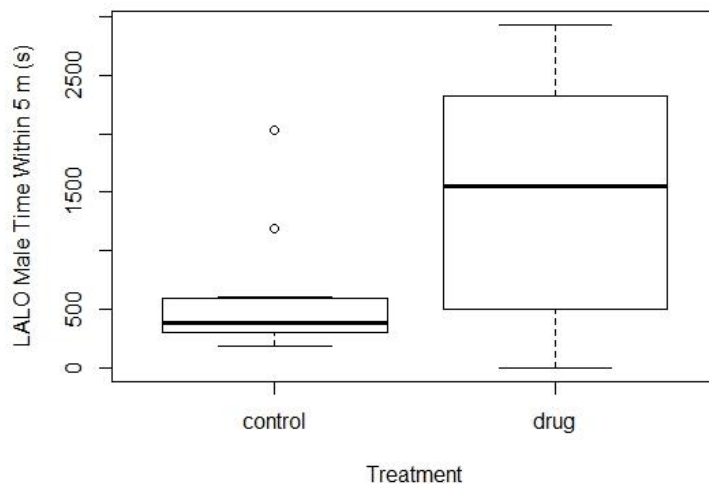nest than controls ($n=11$).

Fig. 5. Male LALO time within 5 m of the nest

The time spent within 5 m of the nest during a 1 h observation period on day six post-hatch for LALO males. Birds receiving the drug (*n*=8) were much more likely to spend time within 5 m of the nest compared to controls (*n*=11).
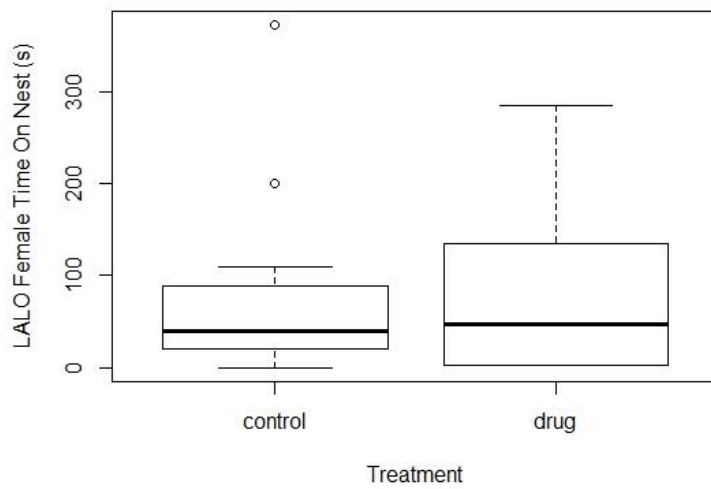
Fig. 6. Female LALO time on nest

Total time spent on the nest by female LALOs during a 1 h observation period on day six post-hatch. There was no difference between groups (control, $n=11$; drug, $n=8$).
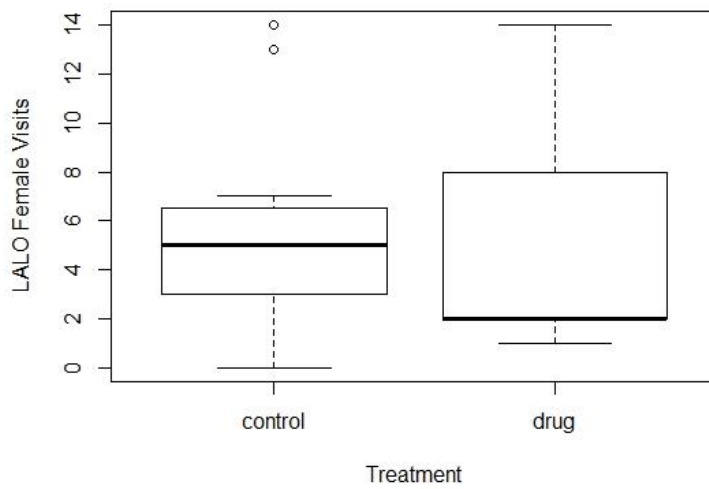
Fig. 7. Female LALO nest visits

Total number of visits to the nest for female LALOs during a 1 h
observation period. I did not observe a difference between groups
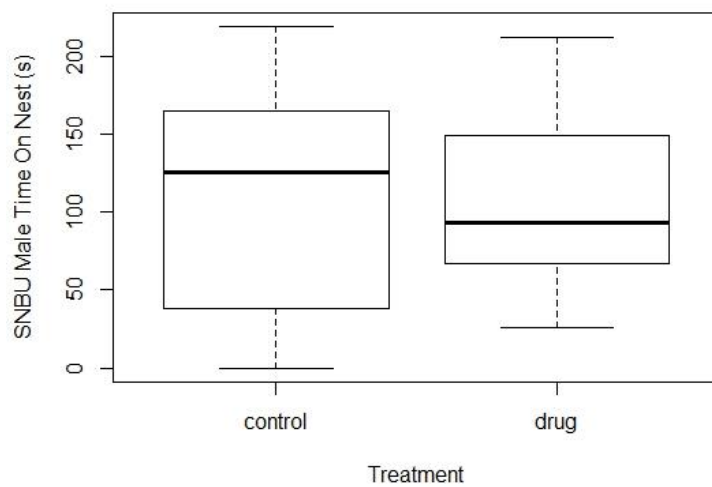(control, *n*=14; drug *n*=10).

Fig. 8. Male SNBU time on nest

The time spent on the nest during a 1 h observation period on day six post-hatch for SNBU males. I observed no significant difference between groups (control, *n=14*; drug, *n=10*).
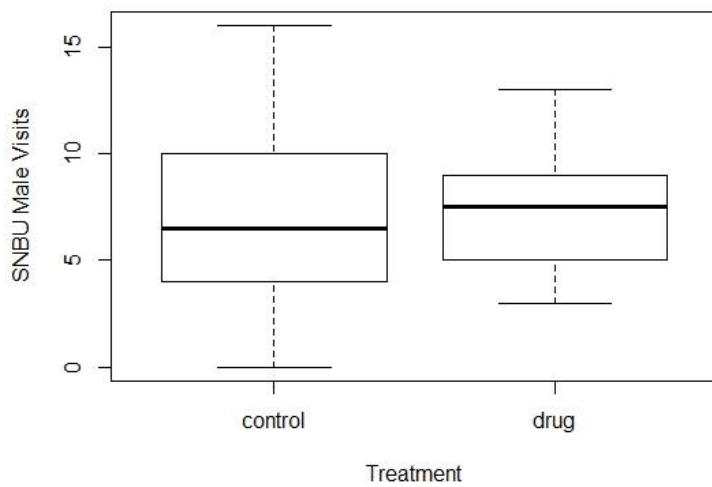
Fig. 9. Male SNBU nest visits

Total number of visits to the nest during a 1 h observation period on day six post-hatch for male SNBUs. I observed no difference in nest visits between groups (control, *n*=14; drug, *n*=10).
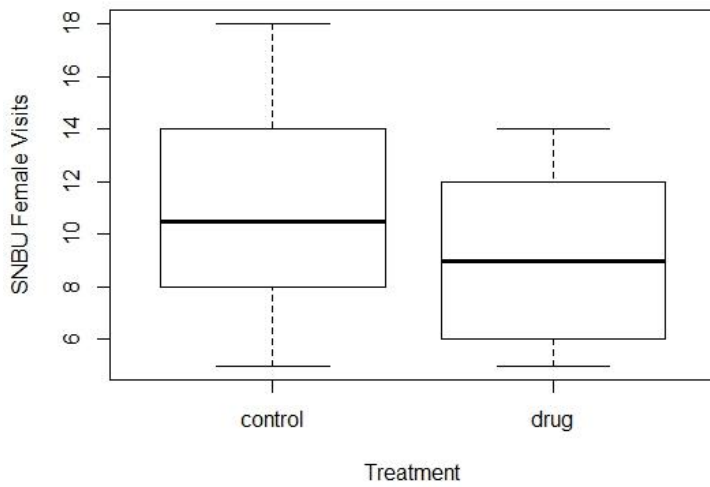
Fig. 10. Female SNBU nest visits

Total number of visits to the nest for female SNBUs during a 1 h observation period on day six post-hatch. I did not observe a difference between groups (control, *n*=11; drug *n*=8).
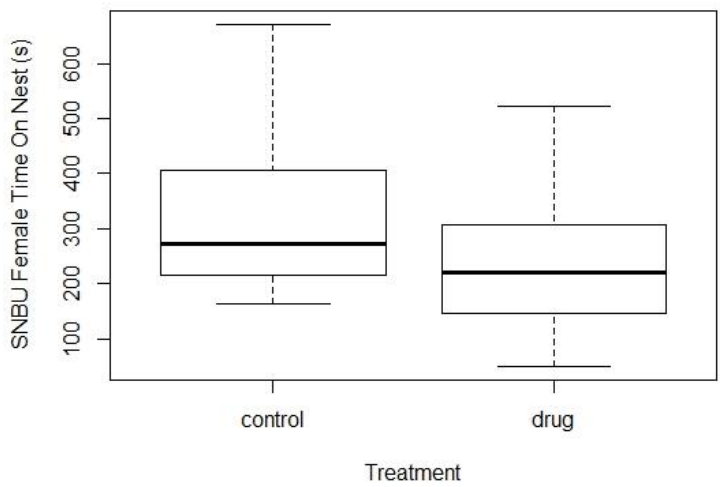
Fig. 11. Female SNBU time on nest

The time spent on the nest during a 1 h observation period on day six post-hatch for SNBU females. I observed no significant difference between groups (control, $n=14$; drug, $n=10$).
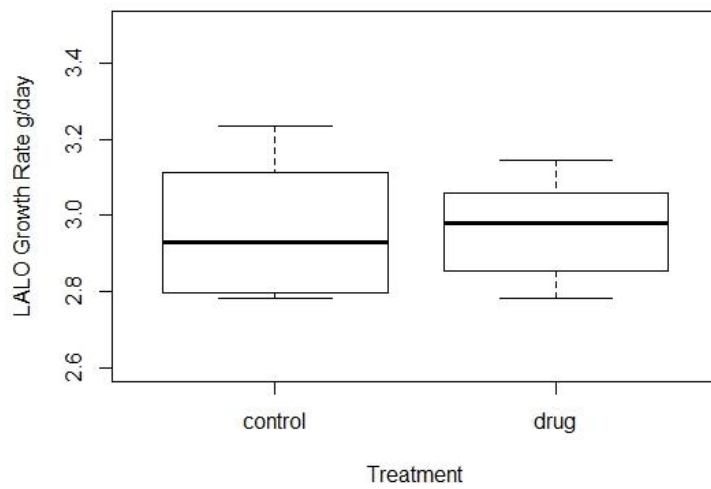
Fig. 12. Growth rate (g/day) for LALO nestlings

Mean Growth rates for each nest in grams per day between day two
and day seven post-hatch. I observed no significant difference in
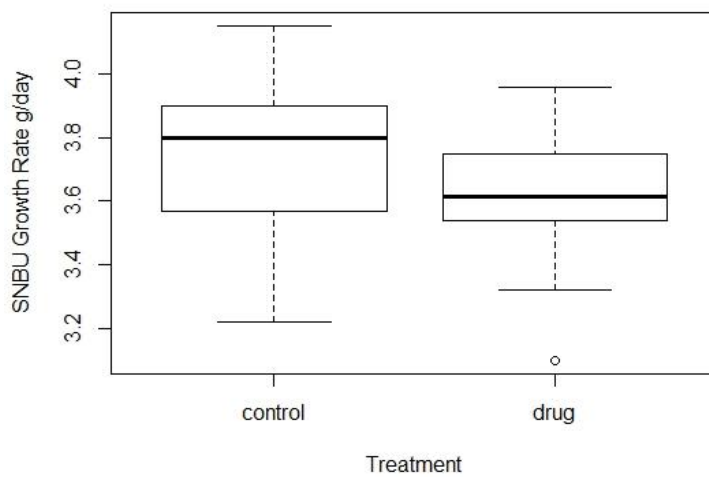growth rates between groups (control, *n=8*; drug, *n=8*).

Fig. 13. Growth rate (g/day) for SNBU nestlings

Growth rates in grams per day between day two and day seven post-hatch. Mean growth rates for each nest were used. I observed no significant difference in growth rates between groups (control, *n*=14; drug, *n*=10).

Table 1. Nestling Growth Rates Day 2 to Day 7

I calculated growth rates for both species in several ways. I found no significance between groups.

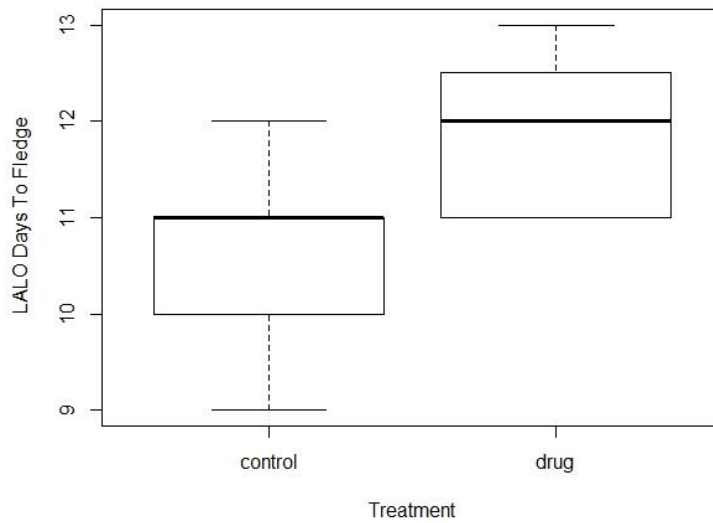| Nestling Growth Rates Day 2- Day 7	SNBU			LALO		
	p	*df*	*t*	*p*	*df*	*t*
Percent Change Nestling Mean	0.076	21.3	1.86	0.303	13.1	1.07
Grams Per Day Nestling Mean	0.229	21.2	1.24	0.558	9.12	-0.609
Percent Change Heaviest Nestling Day 7	0.104	17.5	1.71	0.84	13.5	-0.206
Percent Change Smallest Nestling Day 7	0.228	20.1	1.24	0.7714	13.8	0.296
Highest Growth Rate	0.168	21.5	1.42	0.382	9.56	-0.917

Fig. 14. Days to fledge for LALO nestlings

LALO nests of males that received modafinil (*n*=6) fledged a day
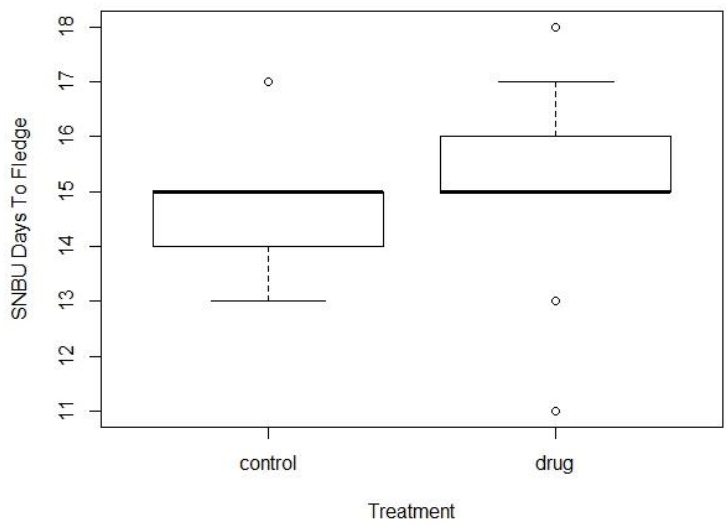later on average compared to controls (*n*=8).

Fig. 15. Days to fledge for SNBU nestlings

There was no difference between groups in time to fledge for
SNBU nestlings (control, *n*=12; drug, *n*=9).

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