Leptin administration does not influence migratory behaviour in white-throated sparrows (*Zonotrichia albicollis*)

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Migratory flights by birds are among the most energetically demanding forms of animal movement, and are primarily fueled by fat as an energy source. Leptin is a critical fat-regulation hormone associated with energy balance in non-avian species but its function in birds is highly controversial. Prior research indicated the effects of leptin differed between birds in migratory condition or not, but no research has assessed the effect of leptin on migratory behaviour itself. In this study, our objective was to determine if leptin affects migratory restlessness and fat deposition in migratory songbirds. We used photoperiod manipulation to induce spring migratory condition, and measured migratory restlessness in leptin-injected and saline-injected white-throated sparrows (*Zonotrichia albicollis*). Leptin treatment had no effect on migratory restlessness nor fat deposition, providing evidence that leptin does not influence avian migratory motivation or behaviour. Our results also further support the idea that birds in a hyperphagic migratory condition may be insensitive to leptin.

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(*Zonotrichia albicollis*)

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Abstract.

17 Migratory flights by birds are among the most energetically demanding forms of animal

18 movement, and are primarily fueled by fat as an energy source. Leptin is a critical fat-regulation

19 hormone associated with energy balance in non-avian species but its function in birds is highly

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23 deposition in migratory songbirds. We used photoperiod manipulation to induce spring migratory

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25 throated sparrows (*Zonotrichia albicollis*). Leptin treatment had no effect on migratory

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27 motivation or behaviour. Our results also further support the idea that birds in a hyperphagic

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Introduction.

The migratory flights of birds are amongst the most energetically demanding activities performed by animals and can involve extended periods of high-intensity exercise with little to no food intake. Birds use fat as their primary metabolic fuel and deposit large amounts of fat in preparation for migration (Dingle, 1996; Jenni & Jenni-Eiermann, 1998; Singh et al., 2018). Depleted fat reserves, and the time it takes to refuel, are major determinants in how long birds remain at stopover sites between bouts of migratory flight (Eikenaar & Schlafke, 2013; Lupi et al., 2019; Goymann et al., 2010). Thus, the regulation of stored body fat is critical to successful migration. Fat reserves and refuelling are controlled by stimulatory and inhibitory circuits in the hypothalamus (Boswell, 2005) under the influence of hormones that signal body condition (Boswell & Dunn, 2015). Two mammalian fat regulation hormones, ghrelin and adiponectin, have been proposed to regulate fat and influence bird migration, including the timing of departure from stopover sites (Goymann et al., 2017; Stuber et al., 2013). However, leptin, an important appetite-regulating hormone in mammals sometimes referred to as the master energy balance hormone, has been less studied with respect to avian migration. In mammals, leptin is secreted from adipose tissue and binds with receptors in the hypothalamus to signal fat deposition. As well, circulating leptin is directly proportional to fat levels (Considine et al., 1996). Thus, with the emergence of leptin's integral role in controlling mammalian fat and energy balance, it is of high interest to determine if it plays a similar role in birds.

Leptin was characterized in birds almost two decades after it was in mammals. This may be attributed to extreme guanine-cytosine content, occasionally referred to as the "dark side of the genome", and low expression levels (Friedman-Einat & Senoussi, 2019). Technological advances in next-generation RNA sequencing resulted in the successful identification of the avian leptin gene (Huang et al., 2014; Friedman-Einat et al., 2014; Prokop et al., 2014). This

54   identification indicated the pattern of genes surrounding the avian leptin gene to be syntenic with

55   those in the mammalian genome. Structural modelling of this avian leptin ortholog revealed a

56   highly conserved hydrophobic core (Prokop et al., 2014). However, an early erroneous leptin

57   sequence identified prior to the emergence of high-depth genome sequencing resulted in over

58   100 papers published, many of which contradict results obtained in those using the correct leptin

59   sequence (Friedman-Einat & Senoussi, 2019). The combination of the delay in discovering a

60   genuine sequence of the avian leptin gene and potentially misleading published papers using an

61   erroneous sequence has resulted in a distinct gap in knowledge on the function of leptin in birds.

62   In theory, avian leptin could be important in the control and success of migration by

63   regulating when birds initiate migration or leave a stopover site based on fat accumulation.

64   However, this mechanism would require avian leptin to function in the same way as its

65   mammalian ortholog. Previous studies have produced conflicting results regarding whether avian

66   leptin correlates with fat reserves and signals this to the brain. Kuo et al. (2005) observed leptin

67   administration resulted in decreased food intake in chickens, and suggested avian leptin may

68   function similarly to its non-avian counterparts. Kordonowy et al. (2009) found leptin acted

69   differently in breeding vs non-breeding birds, and suggested leptin function depends on the

70   breeding state of the bird. Gogga et al. (2013) suggested leptin does not reflect accumulated fat

71   in migratory birds and is therefore not involved in energy balance, nor as a control of body fat.

72   Cerasale et al. (2011) found leptin affected food intake and fat accumulation in wintering, but not

73   migratory sparrows. Thus, while several studies have examined the effects of leptin treatment on

74   birds, there is no consensus as to its function. To add to the complexity of the debate, it appears

75   that leptin's function may vary depending on whether or not a bird is in a hyperphagic migratory

76 state (Cerasale et al., 2011; Zajac et al., 2011). However, there is no information describing the

77 effect of leptin on migratory behaviour itself.

78   Although mammalian and avian leptin have different structures, several studies have

79 found effects of mammalian leptin on bird behavior. Human (Kuo et al., 2005) and murine

80 (Cerasale et al., 2011) leptin affected feeding behaviour in poultry and songbirds, respectively.

81 Murine leptin also affected food-caching behavior in titmice (Henderson et al., 2018). These

82 studies suggest that despite the important differences between mammalian and avian leptin that

83 there may be commonalities.

84   Migratory behaviour can be assessed in captive songbirds using a proxy for the

85 behavioural changes a wild bird would exhibit. Captive birds demonstrate a behaviour called

86 nocturnal migratory restlessness (or *Zugunruhe*), that can include wing whirring and hopping

87 from perch to perch (Agatsuma & Ramenofsky, 2006). The intensity and persistence of

88 migratory restlessness is positively correlated with the probability of birds leaving a site to

89 migrate if they were in the wild (Wingfield et al., 1990; Eikenaar et al 2014). Migratory

90 restlessness is useful to determine migratory condition in captive birds and many studies have

91 found a positive relationship between accumulation of fuel (fat) reserves and migratory condition

92 (Fusani et al., 2009; Eikenaar & Schlafke, 2013, Lupi et al 2016). Prior research has

93 demonstrated that increased levels of appetite-regulating hormones ghrelin and adiponectin both

94 increase migratory restlessness (Goymann et al., 2017; Stuber et al., 2013). This provides further

95 evidence for the role of energy balance hormones in avian fat regulation and migration, and that

96 migratory restlessness can be used as a proxy to assess their role in migratory behaviour.

97   In this study, we induced migratory condition in white-throated sparrows (*Zonotrichia*

98 *albicollis*) with photoperiod manipulation. We administered leptin through a series of injections

99   and used infrared video analysis to measure nocturnal migratory restlessness. We predicted that

100   if leptin is a lipostatic signal in songbirds then sparrows injected with leptin should increase their

101   migratory restlessness. We also measured body composition to assess if leptin affected body fat

102   deposition as the sparrows came into a migratory state. We predicted that sparrows injected with

103   leptin should exhibit higher levels of migratory restlessness, but might potentially deposit less fat

104   if leptin induces negative feedback.

105   **Materials & Methods.**

106   ***Pre-experiment protocol***

107   White-throated sparrows (*Zonotrichia albicollis*) are an abundant short- to medium-

108   distance migrant North American songbird whose migratory restlessness behaviour has been

109   well-characterized, and whose response to leptin has been previously studied (Cerasale et al.,

110   2011; Zajac et al., 2011). We captured 24 white-throated sparrows near Long Point, Ontario,

111   during fall migration in October 2017 (See Figure 1 for experimental timeline). Sparrows were

112   held under a scientific collection permit from the Canadian Wildlife Service (CA-0244) and

113   provided with *ad libitum* access to food and water. Food was a 50/50 mix of Mazuri Small Bird

114   Maintenance (PMI Nutrition International, Brentwood, MO) and Living World Premium Seed

115   Budgie Mix. The University of Western Ontario's Animal Care Committee approved all animal

116   procedures (protocol #2015-055).

117   We held the sparrows in outdoor aviaries at the University of Western Ontario's

118   Advanced Facility for Avian Research until November 2017, at which point we moved them to

119   indoor environmental chambers in individual cages (38 x 38 x 38cm) at 16 °C. Upon bringing

120   sparrows inside the facility in November 2017, we initially maintained photoperiod that

121   mimicked natural conditions.

122   White-throated sparrows are a dimorphic species and are classified as either white-stripe

123 or tan-stripe morphs (Falls & Kopochena, 1994). Sex and morph are both variables that can

124 affect migratory behaviour (Kelly et al., 2020) in white-throated sparrows and so were

125 determined prior to experimentation to balance treatment groups. We collected blood samples of

126 approximately 20 µL via brachial venipuncture that was blotted to sterile filter paper and allowed

127 to dry. We then extracted DNA and amplified regions to determine sex and morph using

128 previously published protocols (e.g., Kelly et al., 2020).

129   Six weeks prior to the experiment, we adjusted the photoperiod to simulate short winter

130 days (8L:16D). We then limited the diet of the sparrows for two weeks prior to the beginning of

131 the experiment to lean the sparrows down to a more realistic pre-migratory condition, and to

132 control for individual leptin concentration levels prior to experimental manipulation. During the

133 experiment, sparrows remained on a fixed diet, but were not food restricted, to control for

134 variation in body condition due to variation in food availability.

135 ***Experimental Protocol***

136   We measured the amount of fat on each sparrow the day before the experiment began

137 (day 0) using a Quantitative Magnetic Resonance (QMR) scanner (Echo MRI-B, Echo-Medical

138 Systems, Houston, TX). This scanner measures fat non-invasively with ±11% accuracy

139 (Guglielmo et al., 2011). On 29 January 2018, we pseudorandomly assigned each sparrow to a

140 treatment group (leptin or saline), balancing for fat deposition, sex, and morph. On this date (Day

141 1 of the experimental period) we photostimulated the sparrows to induce spring migratory

142 behaviour by adjusting the light schedule to mimic the long days of spring (14L:10D) and began

143 a two-week series of daily injections. This photoperiod manipulation reliably induces spring

144 migratory restlessness in this species (Cerasale et al., 2011; Zajac et al., 2011; Vandermeer,

145   2013). Following prior studies (Cerasale et al., 2011; Zajac et al., 2011), we gave each sparrow

146   an intramuscular injection to the pectoral muscle twice per day at lights on and 6 h after, using

147   31-gauge insulin needles. Sparrows in the control group received 0.01 mL PBS per injection (1

148   μg/g body mass; 1 mM phosphate buffer saline, pH ~7.4; Bio Basic Inc., Markham, Ontario,

149   Canada). Sparrows in the experimental group received 0.01 mL murine leptin per injection (1

150   μg/g body mass; murine leptin, Shenandoah Biotechnology Inc., Warwick, PA, USA, purity

151   >95% as measured by RP-HPLC). This dose and treatment has been proven in previous studies

152   assessing behaviour in sparrows (Cerasale et al., 2011; Zajac et al.., 2011). Each bird received

153   the injection in the left pectoral muscle in the morning, and right pectoral muscle in the

154   afternoon. We swabbed the injection site with 70% ethanol to clear feathers from the site before

155   injection.

156   We video recorded the activity of each sparrow from lights off to lights on (10 h per bird)

157   every night from Day 1 of injections to Day 14, and then for a week after cessation of injections

158   (Day 21). Infrared (850 nm) lights (Smart B-Series, model AT-35-B) illuminated the sparrows at

159   night. The infrared lights and video cameras (Black and White High Res Ex View Micro Video,

160   Super Circuits, Austin, Texas, USA) were secured to a metal shelving unit facing the sparrows

161   and connected by wires to two computers set up outside the room to save the recordings in real

162   time each night. The day after the video recordings were completed (Day 22), we measured the

163   fat of each sparrow again to assess changes in fat as the sparrows came into a migratory state.

164   We used Noldus EthoVision XT behavioural software (Noldus Information Technology,

165   Wageningen, NL, www.noldus.com) to quantify the nocturnal migratory restlessness. We

166   created a reference image by editing out the sparrow from a screenshot of each video to be

167   analyzed using Pixlr (PIXLR Materials by Ola Sevandersson, Sweden, available at pixlr.com).

168   EthoVision used the reference image to obtain most accurate noise reduction, and to track

169   movement of the bird that differed from background. Each night recorded as a single trial

170   starting when lights went off and ending the moment before lights came back on (10 h per night).

171   EthoVision measured the time a sparrow spent mobile (s), by quantifying body area fluctuations.

172   We used this time spent mobile as an indicator of migratory restlessness. We also watched the

173   videos to ensure EthoVision accurately measured the bird's behaviour and to confirm the birds

174   displayed nocturnal behaviours characteristic of migratory restlessness: wing whirring and

175   hopping from perch to perch instead of sleeping.

176   ***Statistical analysis***

177   We analyzed the amount of time spent mobile at night (*hereafter migratory restlessness*)

178   using linear mixed models (LMM) in R Studio (v1.1.456). We used migratory restlessness as the

179   dependent variable. This data was heavily-right skewed, so we square-root transformed the data

180   prior to further analysis. Candidate models differed in combinations of trial date, treatment, sex,

181   morph, and trial*treatment. Sex compared male versus female sparrows, while morph compared

182   white-stripe versus tan-stripe plumage morphs. We ran models to assess the effect of leptin on

183   the total experimental period, as well as models focusing on migratory restlessness within the

184   injection vs post-injection recovery phases. All models included a random effect of trial*bird ID

185   to account for non-linear increases in standard error over time. Initially, we constructed 27

186   candidate models to assess the effect of leptin on migratory restlessness over the duration of the

187   entire experiment (Days 1-21), including both the injection and post-injection recovery phase.

188   We then ranked the candidate models based on AICc value. We averaged the top models that

189   were separated by a difference of less than two second-order Akaike's information criterion

190   (AICc). To test for effects of leptin individually in each phase we constructed 17 models

191 separately in each phase: injection (Day 1-14) and recovery (Day 15-21). For both phases, we

192 averaged the top most supported models that were separated by a difference of less than two

193 AICc. An averaged model is a weighted average of parameter estimates where the weights are

194 calculated based on the ΔAIC(c) scores. Our model used conditional averaging, where the slope

195 for the averaged parameters is based only on models that include that parameter. In contrast, full

196 averaging uses a partial slope of 0 for models that do not have a value for a particular parameter.

197 We assessed validity by assessing normality, linearity and equal variance, and leverage. Each

198 model fulfilled the assumptions to be an appropriate model for these data. We assessed changes

199 in body fat composition using a two-way repeated measures ANOVA in IBM SPSS Statistics 27.

200 We used body fat as the dependent variable with time and treatment as the independent variables.

201 **Results.**

202 *Migratory restlessness*

203   Of the candidate models we constructed to assess migratory restlessness in response to

204 leptin administration over the full experimental period (21 days), the averaged top model included

205 the effects of trial, morph, and phase. There was a significant effect of trial date on migratory

206 restlessness ($z = 3.27$, $p = 0.001$; Figure 2). The parameter estimates for morph ($z = 1.80$, $p = $

207 0.060) and phase ($z = 0.575$, $p = 0.565$) were not significantly different from zero. Treatment with

208 leptin was included in the fifth most supported model, but did not have a significant effect on

209 migratory restlessness ($t = -0.196$, $p = 0.845$; Table 1).

210   Of the candidate models we constructed to assess migratory restlessness in response to

211 leptin administration during the injection phase (day 1-14), the averaged top model included the

212 effects of trial, morph, and treatment. There was a significant effect of trial date ($z = 3.40$, $p < $

213 0.001; Table 2) and morph on migratory restlessness ($z = 1.96$, $p = 0.038$). Tan-stripe morph

214 sparrows exhibited higher activity levels than white-stripe morph sparrows for the duration of the

215   experiment. Treatment was included in the averaged top model but the parameter estimate did not

216   significantly differ from zero ($z = 0.488$, $p = 0.623$). In the recovery phase, the single most

217   supported model included the effect of trial date, treatment, morph, and the interaction between

218   trial date and treatment. There was a significant effect of trial date ($z = 2.64$, $p = 0.019$; Table 3).

219   There was no significant effect of treatment ($z = 1.79$, $p = 0.073$), the interaction between trial date

220   and treatment ($z = 1.69$, $p = 0.091$), nor morph ($z = 0.96$, $p = 0.357$).

221   ***Body fat***

222   There was no significant difference in fat between control and leptin injected birds over

223   the course of the experiment ($F_{1,10} = 0.803$, $p = 0.375$; Figure 3). Time did not have a significant

224   effect on fat deposition ($F_{1,10} = 0.046$, $p = 0.831$), nor did treatment ($F_{1,10} = 3.157$, $p = 0.083$).

225   **Discussion.**

226   The endocrine system is critical to the initiation and mediation of avian migration.

227   Hormones like corticosterone and testosterone have been proven to influence migratory activity

228   (Eikenaar et al., 2018; Tonra et al., 2011; Vandermeer, 2013). The role of leptin however, is less

229   clear. The main objective our study was to determine if leptin directly influences the onset of

230   migratory activity in songbirds. While leptin was discovered and characterized in mammals over

231   two decades ago (Zhang et al., 1994), sequencing avian leptin proved to be much more difficult

232   and contentious (Friedman-Einat & Seroussi, 2019). Following the successful identification of an

233   avian leptin ortholog, it then became important to understand the function of the hormone.

234   Mammalian leptin's primary function is to signal fat deposition, and fat deposition is crucial to

235   avian migration initiation. Thus, the idea that avian leptin may influence migratory behaviour is a

236   plausible hypothesis (Cerasale et al., 2011; Gogga et al., 2013).

237   If leptin were to act analogously to non-avian leptin and signal fat deposition, then we

238   expected an increase in disposition to migrate in birds injected with leptin. However, we found

239   no evidence that administered leptin affected migratory disposition. Leptin injection did not

240   increase migratory restlessness, suggesting that increased leptin is not involved in the onset of

241   migratory behaviour in white-throated sparrows. We also found no effect of increased leptin on

242   fat deposition over the duration of our experiment, which we expected if leptin is a lipostatic

243   signal. In particular we expected lower fat levels if leptin administration falsely signalled

244   increased fat deposition, which was not consistent with our results. Saline injected birds also did

245   not accumulate significant increases in fat, thus it is important to note our experiment assessed

246   the initial onset of migration. It is possible 21 days after a photoperiod change is not enough time

247   for birds to deposit significant amounts of fat for migration. It is also possible that the stress of

248   injections or the fixed amount of food provided daily affected fat levels in the birds.

249   It is possible that our null results could be a result of our use of murine leptin in this

250   study, as avian leptin was not available to synthesize at the time. Avian and mammalian

251   predicted protein sequences show low sequence similarity (~30%). The predicted protein

252   sequence of avian leptin also varies among birds (~45%). However, the structure of avian and

253   mammalian leptin binding-domains are highly conserved, with ~80% identical amino acids (Hen

254   et al., 2008; Adachi et al., 2008). To our knowledge, no study has compared the effect of

255   endogenous and heterologous leptins (Friedman-Einat & Seroussi, 2019). Therefore, for our

256   study, murine leptin injections were the same dose and administration regime as in other studies

257   that found effects on songbird behaviour (Cerasale et al., 2011; Zajac et al., 2011). For this

258   reason, it is unlikely that the null results stem from insufficient dosing or heterologous leptins,

259   though further studies would be required to confirm this. It is also possible that leptin treatment

260   closer to nightfall and the onset of migratory restlessness could affect migratory behaviour, but

261   this would require further studies. Our injections during the daytime did not have any detectable

262   direct or indirect effects, but it remains possible that there could be rapid direct effects if birds

263   experienced elevated leptin during the nighttime at the time of migratory behavior.

264   It is worth noting there was a significant effect of plumage morph during the injection

265   phase of the experiment. Morph has previously been shown to affect migratory activity in white-

266   throated sparrows (Kelly et al., 2020). While our study supports this, we also provide evidence

267   that tan-stripe morph sparrows show a higher disposition to initiate migration compared to the

268   white-stripe morph, suggesting that they may advance the onset of spring migration. Although

269   behavioural differences in parenting and territorial singing behaviour between tan-stripe and

270   white-stripe white-throated sparrows are well documented (Falls & Kopochena, 1994), our study

271   adds to evidence that indicate these morphs also differ in migration behaviour.

272   Our results suggest that leptin does not act in a similar role to its non-avian homologues

273   and signal fat deposition in migratory songbirds. This finding conceptually replicates previous

274   work on white-throated sparrows that assessed the role of leptin in foraging, food intake, and fat

275   mass in wintering and migratory birds (Cerasale et al., 2011). Cerasale et al. (2011) found that

276   birds decreased foraging behaviour in response to leptin in the winter, but not when in a

277   migratory status. Further, wintering sparrows injected with leptin lost significantly more fat

278   mass, while migratory sparrows injected with leptin actually increased fat mass. Their results

279   indicated that while leptin may influence behaviour and fat deposition in the winter, there is no

280   effect on birds in a migratory condition. Gogga et al. (2013) also found that leptin did not reflect

281   the extent of fat in migratory dunlins (*Caldris alpine*). Our study supports the view that

282   migratory birds do not respond to leptin as a hormone that regulates fat, or fat accumulation

283   behaviour.

284   Unlike migratory birds, wintering and resident birds appear to respond to leptin. Leptin

285   attenuates food intake in non-migratory birds such as chickens, quail, and titmice (Denbow et al.,

286   2000; Lõhmus et al., 2003; Lõhmus et al., 2006). Further, leptin reduced food-hoarding

287   behaviour during winter in coal tits (*Periparus ater;* Henderson et al., 2018). The fact that only

288   birds in a non-migratory state respond to leptin administration suggests that birds become

289   insensitive to leptin as they come into a migratory state, at a time when overriding leptin

290   signaling would induce hyperphagia to build critical fat reserves necessary for migration.

291   An alternative explanation for our null results is that avian leptin is not an adipokine and

292   therefore not expressed in adipose tissue as birds come into a migratory state (Friedman-Einat &

293   Seroussi, 2019). Quantitative PCR profiling of leptin mRNA in zebra finches (*Taeniopygia*

294   *guttata*) indicated that leptin mRNA was expressed in the brain and pituitary, but not in

295   peripheral tissues including fat (Huang et al., 2014). However, Cerasale et al. (2011) found both

296   leptin receptor isoforms expressed in adipose tissue and the hypothalamus in both wintering and

297   non-wintering birds. Further, they assessed a suppressor of cytokine signalling 3 (SOCS3),

298   which has been proposed as a mechanism involved in leptin resistance. While their data was not

299   consistent with the expected increase in SOCS3 expression associated with leptin resistance,

300   their experiment sampled 40 days after photoperiod stimulation when birds were already in a

301   migratory state which may have missed shorter term effects. It is possible the changes associated

302   with receptor isoform and SOCS3 expression occur transiently after a change in photoperiod.

303   Our experiment allowed us to assess this initial change in migratory behaviour after a change in

304   photoperiod when birds begin to develop a migratory phenotype. Based on our null results in

305   response to leptin, we therefore suggest as birds come into a migratory state, they may not

306   express leptin, nor the receptors, in fat tissues. Furthermore, SOCS3 may increase at the initial

307   onset of the migratory phenotype to assist in leptin resistance to induce hyperphagia. Future

308   research in this area should consider this possibility and further study the regulation of leptin and

309   leptin receptor expression at the onset of migration.

310   **Conclusions.**

311   Our study adds to a growing body of research on the hormonal regulation of avian

312   migration. Prior research suggests that avian leptin does not influence the feeding or foraging

313   behaviour of birds preparing for migration. Here, we provide empirical evidence that increased

314   leptin does not influence fat deposition in songbirds, and that leptin does not directly influence

315   migratory behaviour itself. We suggest that migratory birds are insensitive to increases in

316   circulating leptin, or differentially express leptin to facilitate hyperphagia at the onset of the

317   migratory phenotype. Further study of the function of avian leptin is important to better

318   understand the hormonal regulation of migration.

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Selection of models predicting captive white-throated sparrow migratory restlessness during full 3 week experiment, including both injection and recovery phases.

Note: (A) Top 3 candidate linear models (and null model) predicting the change in migratory restlessness of captive white-throated sparrows after a two week experimental injection phase (leptin or saline), followed by a one week post-injection phase. Reported in the table is the second-order Akaike's information criterion (AICc), difference in AICc between candidate models (AICc), and proportional weight of each model (*w*i). (B) Real function parameters of the best-fitting model predicting migratory restlessness of white-throated sparrows injected with leptin or saline. A higher estimate indicates higher levels of migratory restlessness. Italics indicate traits for which the 95% confidence interval (CI) surrounding the estimate does not overlap with zero.

1   **Table 1. Selection of models predicting captive white-throated sparrow migratory**

2   **restlessness during full 3 week experiment, including both injection and recovery phases.**

	AICc	ΔAICc	w_i
A. Ranked candidate models:			
Trial + Treatment + Morph	4620	0.00	0.203
Trial + Morph + Phase	4621.1	1.15	0.114
Trial + Morph	4621.7	1.74	0.085
Null	4635.2	15.24	0.000

	Estimate	SE	95% CI	p-value
B. Parameter estimates of averaged top models:				
Intercept	80.31	7.19	*66.19 to 94.42*	
Trial	-1.51	0.35	*-2.20 to -0.82*	<0.001
Morph (tan stripe)	16.38	8.70	-0.71 to 33.48	0.060
Phase (post)	-2.47	4.28	-10.89 to 5.95	0.565

4   Note: (A) Top 3 candidate linear models (and null model) predicting the change in migratory

5   restlessness of captive white-throated sparrows after a two week experimental injection phase

6   (leptin or saline), followed by a one week post-injection phase. Reported in the table is the second-

7   order Akaike's information criterion (AICc), difference in AICc between candidate models (Δ

8   AICc), and proportional weight of each model (w_i). (B) Real function parameters of the best-fitting

9   model predicting migratory restlessness of white-throated sparrows injected with leptin or saline.

10   A higher estimate indicates higher levels of migratory restlessness. Italics indicate traits for which

11   the 95% confidence interval (CI) surrounding the estimate does not overlap with zero.

Selection of models predicting captive white-throated sparrow migratory restlessness during experimental injection phase.

Note: (A) Top 3 candidate linear models (and null model) predicting the change in migratory restlessness of captive white-throated sparrows after a two week experimental injection phase (leptin or saline). Reported in the table is the second-order Akaike's information criterion (AICc), difference in AICc between candidate models (AICc), and proportional weight of each model (w_i). (B) Real function parameters of the best-fitting model predicting migratory restlessness of white-throated sparrows injected with leptin or saline. A higher estimate indicates higher levels of migratory restlessness. Italics indicate traits for which the 95% confidence interval (CI) surrounding the estimate does not overlap with zero.

1 **Table 2. Selection of models predicting captive white-throated sparrow migratory**

2 **restlessness during experimental injection phase.**

	AICc		ΔAICc	w_i
A. Ranked candidate models:				
Trial + Morph	3150.7		0.00	0.303
Trial	3152.6		1.84	0.121
Trial + Treatment + Morph	3152.6		1.87	0.119
Null	3160.2		9.50	0.003
	Estimate	SE	95% CI	p-value
B. Parameter estimates of averaged top models:				
Intercept	82.10	7.94	*66.48 to 97.72*	
Trial	-1.83	0.52	*-2.86 to -0.79*	<0.001
Morph (tan stripe)	18.77	9.03	*1.01 to 36.52*	0.038
Treatment (saline)	-4.39	8.99	-22.07 to 13.29	0.623

4 Note: (A) Top 3 candidate linear models (and null model) predicting the change in migratory

5 restlessness of captive white-throated sparrows after a two week experimental injection phase

6 (leptin or saline). Reported in the table is the second-order Akaike's information criterion (AICc),

7 difference in AICc between candidate models (ΔAICc), and proportional weight of each model

8 (w_i). (B) Real function parameters of the best-fitting model predicting migratory restlessness of

9 white-throated sparrows injected with leptin or saline. A higher estimate indicates higher levels of

10 migratory restlessness. Italics indicate traits for which the 95% confidence interval (CI)

11 surrounding the estimate does not overlap with zero.

Selection of models predicting captive white-throated sparrow migratory restlessness during post-injection recovery phase.

Note: (A) Top 4 candidate linear models (and null model) predicting the change in migratory restlessness of captive white-throated sparrows after a two week experimental injection phase (leptin or saline), followed by a one week post-injection phase. Reported in the table is the second-order Akaike's information criterion (AICc), difference in AICc between candidate models (AICc), and proportional weight of each model (*w*i). (B) Real function parameters of the best-fitting model predicting migratory restlessness of white-throated sparrows injected with leptin or saline. A higher estimate indicates higher levels of migratory restlessness. Italics indicate traits for which the 95% confidence interval (CI) surrounding the estimate does not overlap with zero.

1 **Table 3. Selection of models predicting captive white-throated sparrow migratory**

2 **restlessness during post-injection recovery phase.**

	AICc	ΔAICc	w_i
A. Ranked candidate models:			
Trial	1454.3	0.00	0.208
Treatment + Trial + Treatment × Trial	1455.4	1.04	0.123
Trial + Morph	1455.7	1.38	0.104
Null	1456.0	1.64	0.092
Trial + Treatment	1456.1	1.80	0.084

	Estimate	SE	95% CI	p-value
B. Parameter estimates of averaged top models:				
Intercept	-17.11	31.57	-79.43 to 45.20	
Trial	3.99	1.69	*0.65 to 7.32*	0.019
Treatment (saline)	77.46	42.90	-7.31 to 162.22	0.073
Trial × Treatment (saline)	-3.88	2.28	-8.38 to 0.62	0.091
Morph (tan stripe)	9.95	10.71	-11.22 to 31.12	0.357

4 Note: (A) Top 4 candidate linear models (and null model) predicting the change in migratory

5 restlessness of captive white-throated sparrows after a two week experimental injection phase

6 (leptin or saline), followed by a one week post-injection phase. Reported in the table is the second-

7 order Akaike's information criterion (AICc), difference in AICc between candidate models (Δ

8 AICc), and proportional weight of each model (*wi*). (B) Real function parameters of the best-fitting

9 model predicting migratory restlessness of white-throated sparrows injected with leptin or saline.

10 A higher estimate indicates higher levels of migratory restlessness. Italics indicate traits for which

11 the 95% confidence interval (CI) surrounding the estimate does not overlap with zero.

Figure 1

Timeline of experimental procedure and bird care for white-throated sparrows (*Zonotrichia albicollis).*

Top portion indicates key dates associated with experimental injection procedure of either leptin or saline, and nocturnal video recording. Bottom portion indicates key dates associated with bird care including housing and diet. Bolded numbers indicate the date the phase started on.
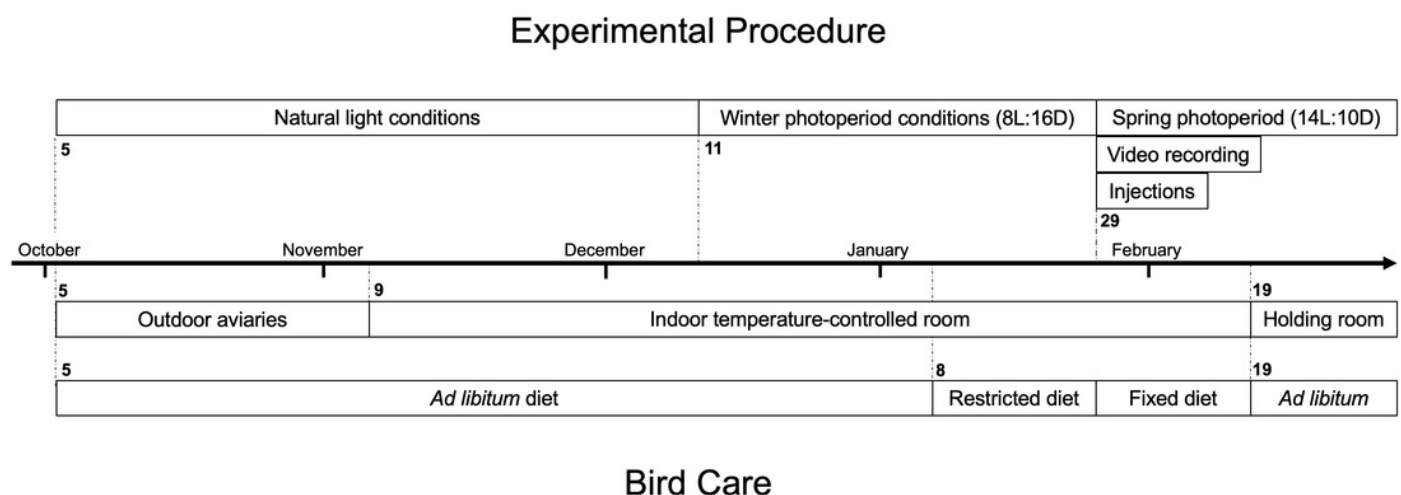
Figure 2

Effects of leptin treatment on migratory restlessness, measured as time spent mobile (minutes), (mean ± SEM) in white-throated sparrows (*Zonotrichia albicollis).*

Sparrows in the experimental group received leptin injections and are denoted by the black squares. Sparrows in the control group received saline injections and are denoted by the open circles. The vertical dotted line indicates the cessation of injections. There was no significant effect of leptin on migratory restlessness over time.
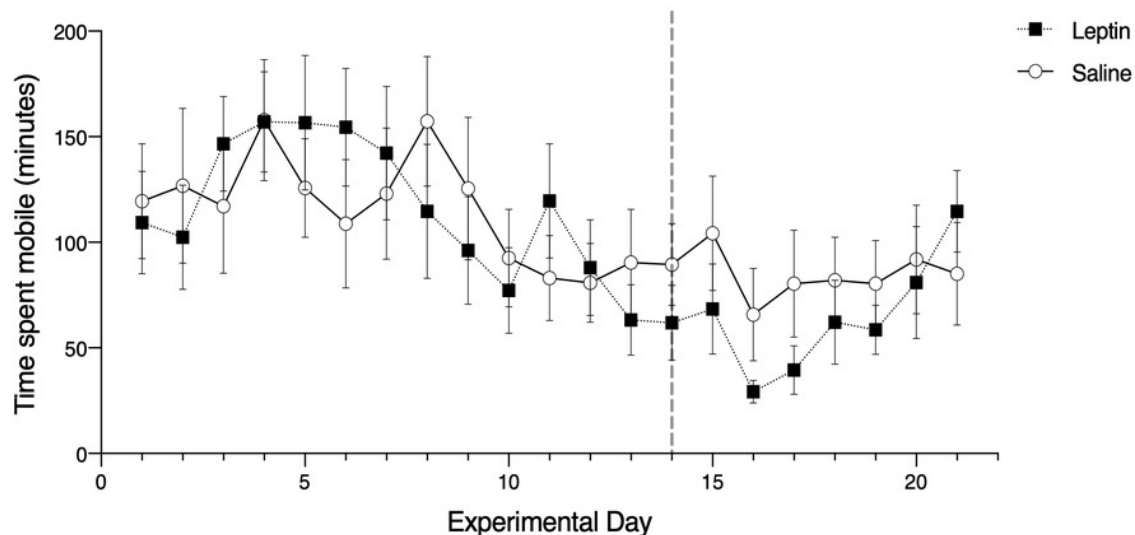
Figure 3

Fat mass (mean ± SEM) of white throated sparrows (*Zonotrichia albicollis)* before (Day 0) and after experimental injections (Day 22).

Sparrows in the experimental group received leptin injections and are denoted by the black squares. Sparrows in the control group received saline injections and are denoted by the open circles. The vertical dotted line indicates the cessation of injections (day 14). There was no significant effect of leptin on migratory restlessness over time.