

ORIGINAL ARTICLE

A glucocorticoid receptor antagonist affects corticosterone but not neophobia in wild-caught house sparrows (*Passer domesticus*)

Marquise S. Henry  | Melanie G. Kimball  | Ella B. Cochran  |
Blake A. Dusang  | William J. Frazier | Keegan R. Stansberry  |
Tosha R. Kelly  | Emily G. Stelling | Christine R. Lattin 

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA

Correspondence

Marquise S. Henry, Department of Biological Sciences, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA 70803, USA.

Email: mhenr55@lsu.edu

Funding information

Louisiana State University; National Science Foundation, Grant/Award Number: IOS-2237423

Abstract

Avoidance of novel stimuli (neophobia) affects how wild animals interact with their environment and may partly determine whether animals persist in human-altered landscapes. The neuroendocrine mediators of neophobia are poorly understood, although past work demonstrated that experimentally reducing circulating corticosterone in wild-caught house sparrows (*Passer domesticus*) decreased neophobia toward novel objects placed near the food dish. In this experiment, we directly tested the role of one of the two types of corticosterone receptors, the glucocorticoid receptor (GR), in mediating neophobia in house sparrows by administering a GR antagonist (RU486, $n = 10$) or a vehicle control (peanut oil, $n = 10$) over 5 consecutive days and measuring responses to novel objects both pre- and post-treatment. We also measured baseline and stress-induced corticosterone in all sparrows on the final day of behavior trials. To better understand the effects of RU486 on corticosterone over time, in a separate group of sparrows ($n = 12$) we administered RU486 or vehicle over 5 days and took multiple blood samples to assess baseline and stress-induced corticosterone. Overall, we did not detect an effect of subcutaneous RU486 injections on neophobia behavior. However, we did find that RU486 injections significantly decreased stress-induced corticosterone levels starting 1 day post-injection and baseline corticosterone levels starting 6 days post-injection, compared to vehicle-injected controls. Our results suggest that GR is not involved in mediating neophobia behavior in house sparrows.

KEYWORDS

HPA axis, neophobia, songbird

1 | BACKGROUND

Neophobia, aversive behavior towards novel stimuli, affects the ability of organisms to adapt to human-altered environments and the novel resources and dangers they contain.^{1–3} A neophobic individual may

not be able to make use of novel foods and shelters, but it may avoid potentially harmful novel situations and predators.^{4–7} As natural environments become more urban and suburban, and roads, cell phone towers, and other human infrastructures crisscross the planet, it has become critical to understand neophobia in wild populations. Despite

Marquise S. Henry and Melanie G. Kimball contributed equally to this work.

the importance of this behavior in helping animals adapt to changing landscapes, the neuroendocrine mechanisms underlying neophobia in wild animals are not well understood.^{8,9} Without an understanding of these mechanisms, our ability to predict the capacity of wildlife to adapt to the Anthropocene is limited.

One potential mediator of neophobia is the glucocorticoid hormone corticosterone, which is released in multiple vertebrate taxa, including birds and rodents, both at baseline and after activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to stressors.^{10–13} Corticosterone can affect behavior in various ways, including an increase in overall activity, aggression, and feeding behaviors.^{14–16} It has been generally hypothesized that corticosterone may affect neophobia, and several studies have found mixed relationships between animals' endogenous baseline or stress-induced corticosterone concentrations and neophobia.^{17–19} Most studies investigating the relationship between glucocorticoids and neophobia in wild animals have typically correlated endogenous glucocorticoids with behavioral responses to novelty. Some studies have found positive associations between corticosterone and neophobia e.g., increased stress-induced corticosterone associated with increased neophobia,^{17,20} while others have found a negative association e.g., decreased baseline corticosterone associated with increased neophobia.^{18,19} Although correlative studies imply a connection between corticosterone and neophobia, they cannot determine the relationship's causality. Also, these studies do not always clearly distinguish whether neophobia should be related to baseline corticosterone, which is more homeostatic in its functions, or to acute corticosterone, released in response to stress.²¹

The findings of studies that experimentally test the relationship between corticosterone and neophobia are more limited and appear somewhat contradictory. For example, when European starlings (*Sturnus vulgaris*) received injections of corticosterone, their response to novel objects near the food dish did not change.²² However, the same study showed that exposure to a stressor that increased corticosterone also increased latency to feed in the presence of a novel object, suggesting a more complicated relationship between neophobia and corticosterone might exist. Another study gave house sparrows (*Passer domesticus*) the drug mitotane, which has been shown to reduce both circulating baseline and stress-induced corticosterone in birds²³; mitotane also reduced neophobia towards novel objects.²⁴ Although these experimental studies convincingly portray corticosterone as a mediator of neophobia behavior, the mechanism by which corticosterone elicits this change remains unknown.

Corticosterone can bind to two different populations of intracellular receptors^{25,26} that elicit different effects on the body: lower-affinity glucocorticoid receptors (GR) and higher-affinity mineralocorticoid receptors (MR).^{27,28} Because of these affinity differences, the MR is occupied at lower plasma glucocorticoid concentrations (i.e., baseline corticosterone), whereas GR typically requires much higher stress-induced concentrations for activation.^{29–31} This makes studying receptors crucial to understanding the relationship between corticosterone and neophobia.

In this study, we specifically tested a GR-related hypothesis: if corticosterone effects on neophobia primarily occur via stress-induced concentrations binding to GR, then blocking GR with an antagonist should decrease neophobia. To test this hypothesis, we

used RU486 (also called mifepristone), a GR antagonist^{26,32} to inhibit GR activation in wild-caught house sparrows. House sparrows show wide and repeatable variation in neophobia in the lab, where some sparrows are reluctant to approach novel objects and eat novel foods, and others are not.^{33–35} This makes them an excellent study species for examining the effects of GR manipulation on neophobia. We tested neophobia in response to novel objects presented with familiar food dishes before and after sparrows received doses of RU486 or a vehicle control. Because RU486 only binds GR, no change in neophobia with exposure to this drug would suggest that corticosterone's effects on neophobia occur via MR activation. To better understand the effects of RU486 on corticosterone over time, we also collected baseline and stress-induced blood samples in a separate group of birds over 5 days of treatment with either RU486 or a vehicle control.

2 | METHODS

2.1 | Study subjects

House sparrows (RU486 + neophobia study: $n = 9$ males and $n = 11$ females, RU486 + corticosterone study: $n = 5$ males and $n = 7$ females) with adult plumage were caught in East Baton Rouge and Iberville Parishes using mist nets at baited feeders in February–April 2023 and September 2023 for the neophobia study, and March–April 2024 for the corticosterone study under Louisiana State Scientific Collecting Permits. Sparrows were solo housed at the Louisiana State University vivarium, with ad libitum access to water, mixed seeds, grit, and a vitamin-enriched food supplement (Mazuri Small Bird Diet). Sparrows could hear but not see other individuals to prevent them from observing novel objects or conspecific responses to novel objects, as one sparrow's behavior could be affected by observing the responses of others.³⁶ Cages (56 cm length $\times$ 45 cm width $\times$ 33 cm height) were equipped with multiple perches, a plastic evergreen branch as a hide, and a sand dish for dustbathing (the latter being removed during novel object trials). During both studies, sparrows were housed in two separate rooms in the animal facility to accommodate blood sampling logistics. Both rooms were kept at light cycles that reflected capture date, and all individuals given at least 3 weeks to acclimate to captivity before studies began. Methods of capture, transport, husbandry, injections, and blood sampling were based on the Ornithological Council's Guidelines to the Use of Wild Birds in Research,³⁷ and all procedures were approved by Louisiana State University Institutional Animal Care and Use Committee, project #56-2022. With blood sampling, in no case did the total amount of blood drawn exceed 1% of body mass at any one time point, or 1.5% of body mass over a two-week period. At the end of this project, sparrows were included in a different experiment, so they were not euthanized as a part of this study.

2.2 | Study design and GR manipulation

The RU486 + neophobia study took place over two consecutive weeks. The first week involved 5 days of neophobia trials without

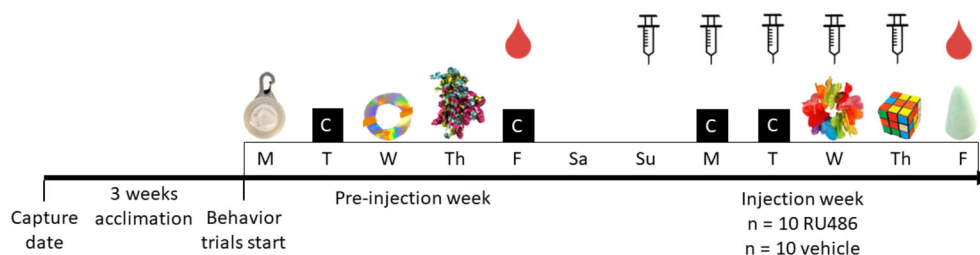


FIGURE 1 Schedule for the RU486 + neophobia study, which involved neophobia behavior trials, injections, and blood sampling. The schedule shown is an example from one individual sparrow, with the order of novel object days (novel objects with food; $n = 3$ per week) and control days (food alone; $n = 2$ per week) randomized each week. Novel objects were placed in, on, around, or over the food dish. All individuals ultimately saw all six novel objects; the order of objects was also randomized (depiction is an example). Objects were a blinking white light, holographic cover, curly ribbon, rainbow lei wristlet, mini Rubix cube, and teal clay cone. Control trials are represented by a black box with a “C”. Red drops indicate days when we collected blood samples to measure baseline and stress-induced corticosterone (2 days before injections and on the sixth-day post-injection). Syringes indicate days sparrows received RU486 ($n = 10$) or vehicle ($n = 10$) injections.

injections (hereafter: pre-injection week) and the second involved another 5 days of neophobia trials with RU486 (or vehicle control) injections administered the morning following behavior trials (hereafter: injection week; Figure 1). Behavior trials ended at 0830 AM, and injections were given at 0900 AM. During the injection week, $n = 10$ sparrows received 100 μ L subcutaneous injections of RU486 over the pectoralis (M8046, Sigma Aldrich, Burlington, MA) sonicated in peanut oil (50 mg/kg), and $n = 10$ sparrows received 100 μ L subcutaneous injections of peanut oil alone as a vehicle control. Previous studies using this dose of RU486 subcutaneously have been successfully used in birds to block GR activity in a songbird and a shorebird.^{32,38} RU486 is specific to GR and does not bind to MR in house sparrows.²⁵ While RU486 also acts as a progesterone receptor antagonist in mammals, little work has been done to confirm this in birds. However, radiolabeled progesterone binding to cytosol from chicken oviduct could not be displaced with excess RU486, suggesting that RU486 may not be a progesterone receptor antagonist in birds.^{39,40}

After the final day of neophobia trials each week, blood samples were taken from all individuals to assess baseline and stress-induced corticosterone after behavior trials ended. Sparrows were undisturbed for at least 3 h, a time shown to allow corticosterone concentrations to return to baseline,⁴¹ were caught within 3 min of entering the room, and ~ 60 μ L of blood was collected from the alar vein to assess baseline levels of corticosterone.⁴² Sparrows were then restrained in clean, breathable cloth bags for 30 min and another ~ 60 μ L of blood was collected to assess stress-induced corticosterone levels.^{43,44}

For the RU486 + corticosterone study, another group of sparrows received 5 days of either RU486 ($n = 6$) or vehicle control ($n = 6$) injections as described above but did not undergo neophobia trials, as restraint protocols for sampling stress-induced corticosterone can affect neophobia behavior in birds.²² Baseline and stress-induced blood samples of ~ 30 μ L were collected 1 week before the first RU486 injection and at least 3 h post-injection on days one, three, and five following subcutaneous injections of RU486 or a vehicle at the doses described above (Figure 2).

2.3 | Corticosterone quantification

Blood samples were stored on ice following collection. Plasma was separated from red blood cells by centrifuging at 5000 g for 8 min and then stored at -80°C until assay. The amount of corticosterone in each sample was quantified using enzyme-linked immunoassays (ELISAs) that have been validated for house sparrows (Detect-X Corticosterone Enzyme Immunoassay Kits, K014-H1, Arbor Assays, Ann Arbor, MI).²⁴ Cross-reactivity of RU486 with this ELISA was calculated to be $<0.001\%$ following the methods used in Krasowski et al.⁴⁵ We followed manufacturer instructions, except that 5 μ L plasma samples first underwent triple ethyl-acetate extractions and were left to evaporate overnight in a fume hood. The remaining corticosterone was then reconstituted in 125 μ L of assay buffer the following morning, and samples were vortexed before adding the samples, in random order, to a 96-well assay plate in duplicate. Inter-plate variability was determined using the coefficient of variation of a standard pool of house sparrow plasma run on each plate and averaged 10% overall. Intra-plate variability was determined by calculating the coefficient of variation for all duplicate samples on each plate; this averaged 7%. The sensitivity of this assay was 20.9 pg/mL, as determined by the manufacturer.

2.4 | Neophobia trials

In the RU486 + neophobia study, sparrows underwent five behavior trials each week, two of which were control trials (i.e., the food dish alone with no object present) and the other three of which were novel object trials (i.e., the food dish presented with a novel object; Figure 1). Novel objects used included a blinking white light clipped over the dish, a miniature Rubik's cube presented in the dish, a holographic cover over part of the dish, a lei wristlet around the dish, a curly bow taped to the front of the dish, and a teal clay cone in the dish. This variety of objects was chosen to maximize the range of colors, textures, and shapes of the novel objects.⁴⁶ All novel objects were placed on, above, or inside the food dish, and sparrows saw each

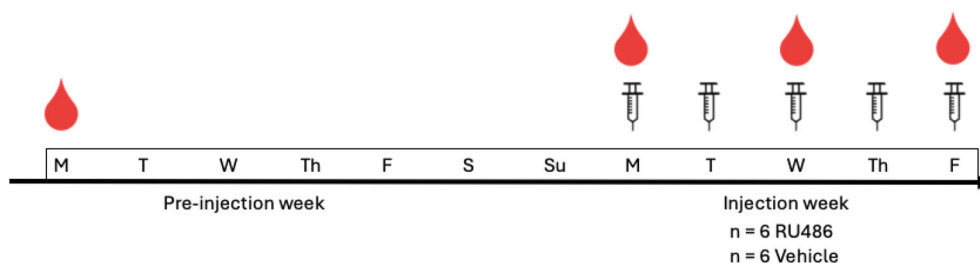


FIGURE 2 Schedule for the RU486 + corticosterone study, which involved RU486 and vehicle injections and blood sampling. Syringes indicate days sparrows received RU486 ($n = 6$) or vehicle ($n = 6$) injections. Red drops indicate days when we collected blood samples to measure baseline and stress-induced corticosterone (on days 1, 3, and 5 after injections); all blood sampling occurred at least 3 h after injections.

object only once. The order in which sparrows encountered these objects and the order of object and control treatments were randomly determined each week for each individual. All objects had previously been shown to increase sparrows' latency to feed compared to control trials.^{24,34,36,47} Each evening prior to trials, food dishes were removed from cages 30 min before lights off, and traces of seeds in the bottom of the cages were removed. This fasting period standardized the motivation of sparrows to approach the food dish to feed during the trials. The following morning, 30 min after lights were turned on, researchers entered the room, replaced food dishes with their individual treatments (object or control), began recording videos of all sparrows (pole-mounted ZOSI Z18.5.Y.2 security cameras directed to a DVR), and left the room for 1 h trials. After this hour was complete, recordings were stopped, and objects were removed from the food dishes. Sparrows do not eat in the lab once the lights are out,⁴⁸ so this represents at most an additional 2 h without food for birds that do not feed during behavior trials. Videos were scored for the time it took (latency) for each sparrow to approach and feed from their food dish by observers blind to treatment. Assessment of this behavior has been consistent between observers in previous studies.⁴⁹

2.5 | Statistical analyses

All statistical analyses were performed in R Studio version 4.3.0.⁵⁰ To determine if RU486 injections affected plasma corticosterone concentrations during the week of neophobia trials in the RU486 + neophobia study, we used a linear mixed effects model from the lmerTest package using the "lmer" function.⁵¹ This model included sparrow ID and ELISA plate as random effects, corticosterone concentration as the dependent variable, and sample type (baseline or stress-induced), week (pre- or post-injections), and treatment (vehicle or RU486) as fixed effects. Before the model was run, a Shapiro-Wilk test was conducted to assess if the data were normally distributed using the "Shapiro.test" function from v.1.3.0 of the tidyverse package.⁵² Because the data were not normal, the "bestNormalize" function from the bestNormalize package was then used to determine the appropriate transformation for the dataset.⁵³ An ordered quartile transformation was used on these plasma corticosterone data before modeling. Next, the effects of RU486 on plasma corticosterone

concentrations in the RU486 + corticosterone study were analyzed using a linear mixed effects model that included sparrow ID and ELISA plate as random effects, sample type (baseline or stress-induced), the day blood was drawn (pre-injection, and days 1, 3, and 5 post-injection), and treatment (vehicle or RU486) as fixed effects, and corticosterone concentration as the dependent variable. Because these data were also not normally distributed, an order of normality transformation was used on plasma corticosterone data prior to modeling. When appropriate, Tukey's post-hoc tests were used with both linear mixed effects models using the "emmeans" function from the emmeans package.⁵⁴ Data were visualized using the "ggplot" function from the ggplot2 package.⁵⁵

For the RU486 + neophobia study, behavioral responses to neophobia trials were analyzed using Cox proportional hazard models with the "coxme" function in the coxme package.⁵⁶ We created five total Cox proportional hazard models, where effect sizes are reported as hazard ratios. A hazard ratio >1 indicates that sparrows fed faster (e.g., during object trials compared to controls). 95% confidence intervals are included for all hazard ratio estimates. All models included trial number as a covariate to control for the effect of repeated behavior trials e.g., to control for generalization,⁵⁷ and potential time course effects of RU486 injections. The first behavioral model confirmed that the novel objects increased feed latency compared to control trials and included object type or controls as a fixed effect, trial number as a covariate, latency to feed as the dependent variable, feed status (fed vs. did not feed) as a censoring variable, and sparrow ID as a random effect. The second model assessed whether different novel objects affected feed latencies differently (i.e. if one object caused higher feed latencies than another) and included object type as a fixed effect, feed latency as the dependent variable, feed status as a censoring variable, and individual ID as a random effect. Pairwise comparisons were performed using the "pairwise_survdif" command in the survival package,⁵⁸ which uses the Benjamini-Hochberg procedure to control the false discovery rate. The third model assessed whether the RU486 and vehicle groups had different responses to novel objects during the pre-injection control week, because the neophobia of individuals was unknown prior to making treatment groups. Thus, this third model used novel object trials from week one only and included treatment (RU486 vs. vehicle) as the fixed effect, trial number as a covariate, latency to feed as the dependent variable, feed status as a censoring variable, and sparrow ID as a random effect.

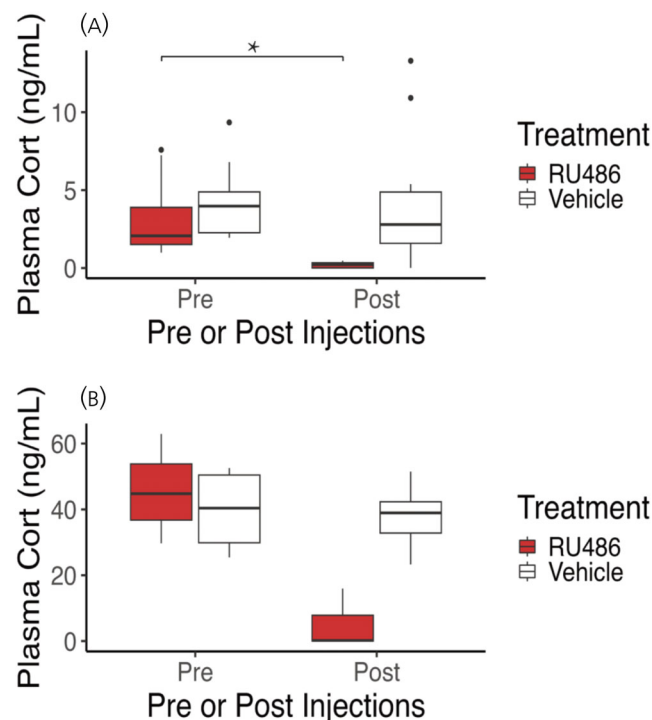


FIGURE 3 In the RU486 + neophobia study, house sparrows' baseline (A) and stress-induced (B) corticosterone concentrations were significantly lower on the sixth day after receiving RU486 ($n = 10$) but not vehicle ($n = 10$) injections. Bars represent standard errors; stars represent significant differences between groups at $p < .05$. Circles indicate samples that were >1.5 times the interquartile range beyond the box. Note that this figure presents raw corticosterone concentrations, but data had to be transformed to meet the assumptions of normality for the statistical analysis.

Fourth, we created a Cox proportional hazard model to directly test our prediction that blocking GR would affect neophobia. This model included treatment, week, a treatment $\times$ week interaction, and trial number as fixed effects; individual ID as a random effect; feed status as a censoring variable; and latency to feed in the presence of novel objects from both weeks of trials as the dependent variable. This model was repeated with control trial data (fifth model) to confirm that any effects of RU486 were specific to novel objects and not related to feeding more generally. We checked that all data met the assumptions of proportional hazards^{59,60} by correlating scaled Schoenfeld residuals with transformed time using the “cox.zph” function in the coxme package,⁵⁶ which were then visualized using the “plot” function. All data met the assumptions (all $X^2 < 1.27$, all $p > .26$). Neophobia was visualized with Kaplan–Meier survival curves using the “survfit” command in the survival package⁵⁸ and the “ggsurvplot” command in the survminer package.⁶¹

3 | RESULTS

3.1 | Corticosterone

In the RU486 + neophobia study, we found a significant three-way interaction of treatment by corticosterone sample by time ($df = 54$,

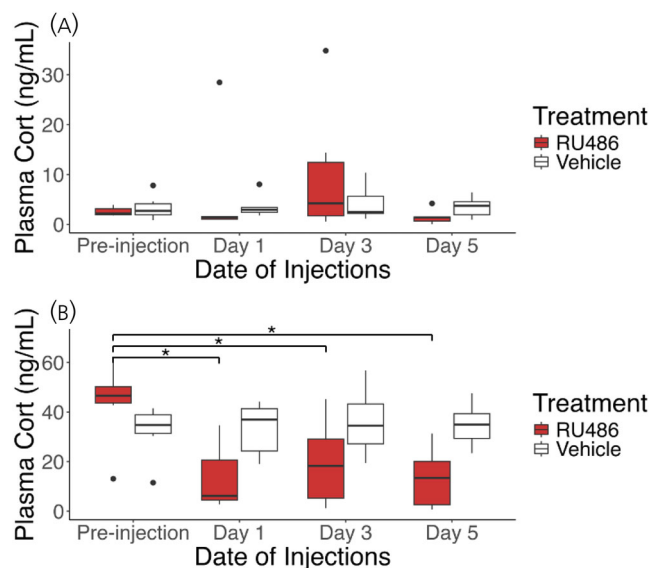


FIGURE 4 Baseline (A) and stress-induced (B) corticosterone concentrations of house sparrows in the RU486 + corticosterone study before (pre-injection) and after receiving either vehicle ($n = 6$) or RU486 ($n = 6$) injections for 1, 3, and 5 days. (A) There were no significant changes in baseline corticosterone concentrations due to treatment or time. However, (B) the GR antagonist RU486 significantly decreased stress-induced corticosterone concentrations in house sparrows after 1, 3, and 5 days of injections. Vehicle injections did not affect stress-induced corticosterone concentrations. Bars represent standard errors; stars represent significant differences between groups at $p < .05$. Circles indicate samples that were >1.5 times the interquartile range beyond the box. Note that this figure presents raw corticosterone concentrations, but data had to be transformed to meet the assumptions of normality for statistical analyses.

$p = .010$, $F = 7.07$, Figure 3). Blocking GR with RU486 reduced baseline corticosterone concentrations on the sixth day (the only sampling point, following behaviour trials/only) post-injection compared to the vehicle control group ($df = 68.90$, $p = .019$, $t = -3.48$; baseline corticosterone mean $\pm$ standard error for RU486 = 0.20 ± 0.06 ng/mL, vehicle = 4.22 ± 1.42 ng/mL; Figure 3A). Stress-induced corticosterone was also reduced on the sixth day after RU486 injections compared to sparrows that received vehicle injections ($df = 69.60$, $p < .001$, $t = -6.17$; stress-induced corticosterone mean $\pm$ standard error for RU486 = 4.02 ± 1.98 ng/mL, vehicle = 37.58 ± 2.60 ng/mL, Figure 3B). Within treatment groups, both baseline and stress-induced corticosterone were decreased on the sixth day after RU486 injections compared to pre-injection values (baseline: $df = 54.4$, $p = .0097$, $t = -3.75$; stress-induced: $df = 53.1$, $p < .0001$, $t = -9.25$, Figure 3A,B), but there was no change in corticosterone over time in the group that received vehicle injections (baseline: $df = 53.3$, $p = .99$, $t = -0.70$; stress-induced: $df = 53.6$, $p = .99$, $t = -0.79$).

In the RU486 + corticosterone study, there were no significant effects of day ($df = 3$, $p = .06$), treatment ($df = 1$, $p = .96$), or a treatment $\times$ day interaction ($df = 3$, $p = .25$; Figure 4A) on baseline corticosterone concentrations over the 5 days of injections. For

TABLE 1 Results of Cox proportional hazard models of house sparrow feeding probability for (A) each novel object type contrasted with control trials during the first week of trials, (B) the effect of treatment and trial number on feed latency during object and (C) control trials during the first week of trials, and (D) the effects of treatment, week, trial number, and treatment $\times$ week on feeding latency during object and (E) control trials. Individual ($n = 20$) was included as a random effect, feed status (fed vs. did not feed) as a censoring variable, and time to feed as the dependent variable.

Fixed effect	β coefficient	Hazard ratio (95% confidence interval)	z-score	p
(A) Novel object and trial number effects (compared to food dish alone)				
Curly bow	-2.65	0.07 (0.21-0.02)	-4.79	<.0001
Holographic cover	-2.50	0.08 (0.24-0.03)	-4.60	<.0001
Rainbow lei	-3.87	0.21 (0.10-0.00)	-5.00	<.0001
Rubix cube	-3.09	0.05 (0.17-0.01)	-4.57	<.0001
Teal cone	-1.94	0.14 (0.35-0.06)	-4.24	<.0001
White light	-1.95	0.14 (0.36-0.06)	-4.17	<.0001
Trial Number	0.41	1.50 (1.83-1.24)	4.09	<.0001
(B) Effect of treatment (RU486 vs. vehicle control) and trial number on feeding latency during novel object trials in the pre-injection week				
Treatment	0.16	1.17 (1.99-0.69)	0.77	.44
Trial Number	0.19	2.20 (1.43-1.01)	3.36	.0008
(C) Effect of treatment (RU486 vs. vehicle control) and trial number on feeding latency during control (food only) trials in the pre-injection week				
Treatment	-0.26	0.77 (1.93-0.31)	-0.56	.57
Trial Number	0.42	1.52 (2.01-1.14)	2.87	.0041
(D) Effect of treatment (RU486 vs. vehicle control), week (pre-injection vs. injection), trial number, and treatment $\times$ week interaction on feeding latency during novel object trials				
Treatment	0.60	1.83 (9.84-0.34)	0.70	.48
Week	-1.96	0.14 (0.51-0.04)	-2.97	.003
Trial Number	0.47	1.60 (1.98-1.29)	4.27	<.0001
Treatment $\times$ Week	-0.30	0.74 (2.28-0.24)	-0.52	.60
(E) Effect of treatment (RU486 vs. vehicle control), week (pre-injection vs. injection), trial number, and treatment $\times$ week interaction on feeding latency during control (food only) trials				
Treatment	-0.14	1.15 (4.69-0.15)	0.16	.87
Week	0.13	1.14 (4.06-0.32)	0.20	.84
Trial Number	0.26	1.30 (1.55-1.09)	2.80	.0051
Treatment $\times$ Week	-0.28	0.76 (2.05-0.28)	-0.54	.59

stress-induced corticosterone, there was no overall significant treatment effect ($df = 1$, $p = .080$). However, we did find a significant day effect ($df = 3$, $p < .001$) and treatment $\times$ day interaction ($df = 3$, $p = .0006$; Figure 4B). RU486 decreased stress-induced corticosterone concentrations after one ($p = .001$, $t = 4.71$), three ($p = .003$, $t = 4.49$), and five ($p = .0002$, $t = 5.333$) days of injections (Figure 4B). There were no significant effects of vehicle injections on stress-induced corticosterone concentrations (all $p > .05$).

3.2 | Behavior

Compared to control trials with the food dish only, all novel objects significantly increased sparrows' latency to feed (all $z < -4.16$, all $p < .01$; Table 1A, Figure 5), and feed latencies did not significantly differ between different objects (Pairwise Log Rank Test, all $p > .12$). There were no differences in feeding latencies between the RU486 and vehicle groups during novel object ($z = 0.58$, $p = .57$, Table 1B) or

control trials ($z = 0.58$, $p = .57$; Table 1C) in the first week of trials; therefore, we ran between-group comparisons. Overall, we saw no effect of RU486 on neophobia behavior (Table 1D). There was a significant effect of trial number ($z = 4.27$, $p < .0001$) and week ($z = -2.97$, $p = .003$) on latency to feed, but no significant effects of treatment ($z = 0.70$, $p = .48$) or a week by treatment interaction ($z = -0.52$, $p = .60$). Sparrows became less neophobic as the experiment progressed, but this decrease in latency to feed was independent of treatment (Figure 6). RU486 injections also did not affect feeding latency during control (food only) trials, as shown by no significant week by treatment interaction ($z = -0.54$, $p = .59$; Table 1E).

4 | CONCLUSION

Our goal in this study was to investigate whether GR in the brain mediates the previously observed effects of corticosterone on neophobia in house sparrows. Based on this hypothesis, we predicted

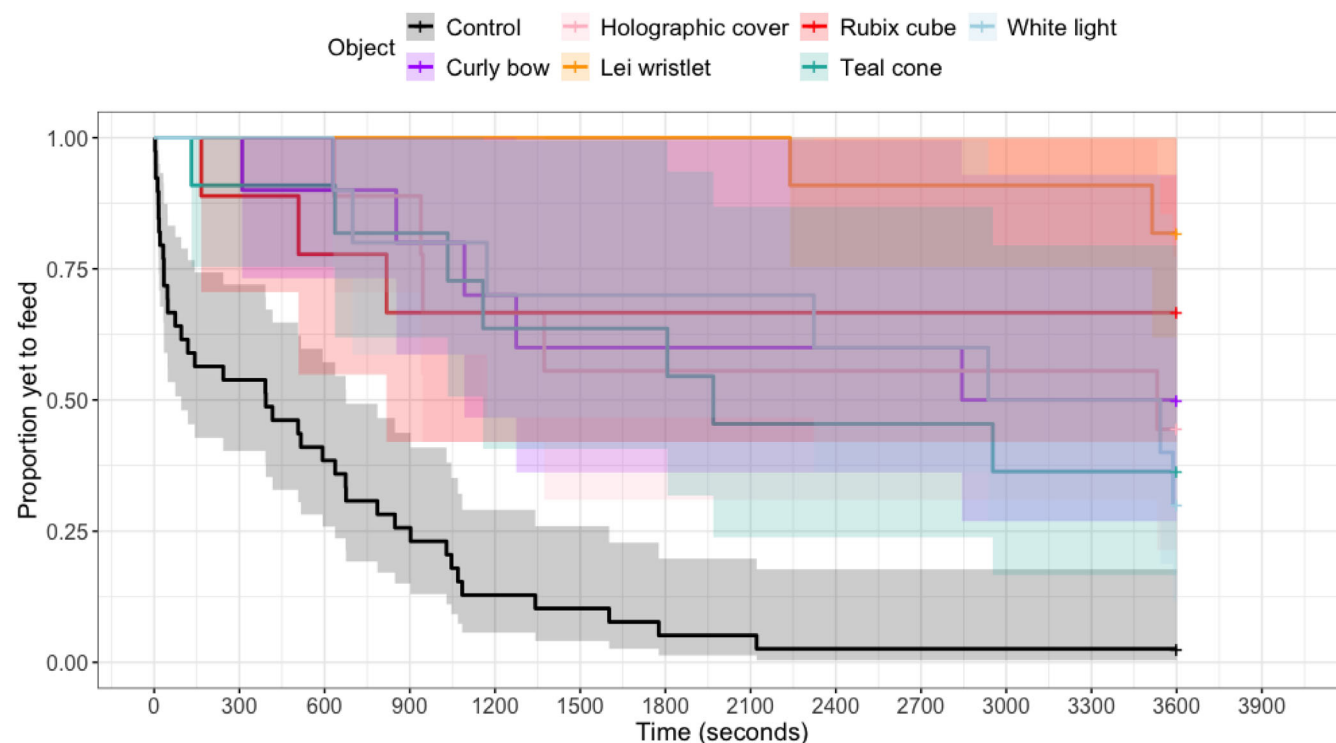


FIGURE 5 All novel objects increased house sparrows' average feeding latencies compared to control trials where the normal food dish was presented (two control trials and three novel object trials were presented to $n = 20$ sparrows each week for 2 weeks, so $n = 80$ control trials and $n = 20$ trials for each novel object). Kaplan–Meier survival curves of average house sparrow feeding likelihood in the presence of a novel object (colored solid lines) or the regular food dish as a control (black solid line). Shaded ribbons represent 95% confidence intervals.

that blocking GR using daily injections of RU486 would decrease neophobia. Instead, we found no effect of RU486 on sparrows' latency to feed in the presence of novel objects. These results suggest that the behavioral response to novel stimuli is not influenced by GR in house sparrows. This is consistent with a study in European starlings where corticosterone injections in the stress-induced range (which binds GR) did not alter responses to novel objects compared to vehicle control injections.²² Additionally, a previous study in house sparrows found no correlation between unmanipulated stress-induced corticosterone and latency to feed in the presence of novel objects,²⁴ further suggesting that stress-induced corticosterone acting via GR does not mediate neophobia. Our results are also consistent with studies in lab rodents that show no effects of RU486 on behavioral responses related to novel stimuli, including responses to novel objects in an open field⁶² and hypophagia in a novel spatial environment.⁶³

Instead, it is possible that sparrow neophobia may be mediated by the effects of higher-affinity MR, which typically shows binding at baseline corticosterone concentrations. Indeed, some studies have found significant correlations between baseline corticosterone and neophobia, although the direction was opposite to what we would expect (i.e., lower baseline corticosterone in more neophobic individuals).^{18,19} One rodent study also found effects of MR antagonists on reactivity to spatial novelty.⁶² It is also possible that corticosterone's effects on neophobia may be driven by permissive effects of corticosterone acting via MR on the sympathetic nervous system

(e.g., epinephrine and norepinephrine).^{64,65} Relationships between norepinephrine and neophobia have been seen in rodents.^{66,67} Further research is needed to determine if MR affects neophobia in wild sparrows and birds generally; unfortunately, to our knowledge, no MR antagonists have been validated for use in songbirds. For example, RU28318 and canrenic acid, two compounds that bind to mammalian MR, did not bind the high-affinity MR-like receptor in house sparrows.⁶⁸

Other possible explanations for our negative results are that RU486 did not penetrate the blood–brain barrier sufficiently to affect brain GR in birds, or that this drug was metabolized too quickly to impact behavior several hours after injection. However, mammal work has shown that even though RU486 has low blood–brain barrier penetration, it still enters the brain and blocks GR-mediated changes in gene transcription.^{69,70} Although RU486 levels in plasma degrade rapidly, on the time scale of 90 min in rats, they persist for much longer in the brain and are still elevated 3 h post administration.⁷¹ In our study, we measured behavior ~22 h after injection to avoid handling birds before behavior trials; therefore, it is possible that we missed the window where we would have seen an effect of RU486 on neophobia.^{64,65}

Although we found no evidence that GR influences neophobia behavior, RU486 injections did reduce house sparrows' baseline and stress-induced corticosterone levels on the sixth-day post-injection. This was an unexpected result given previous studies in songbirds and

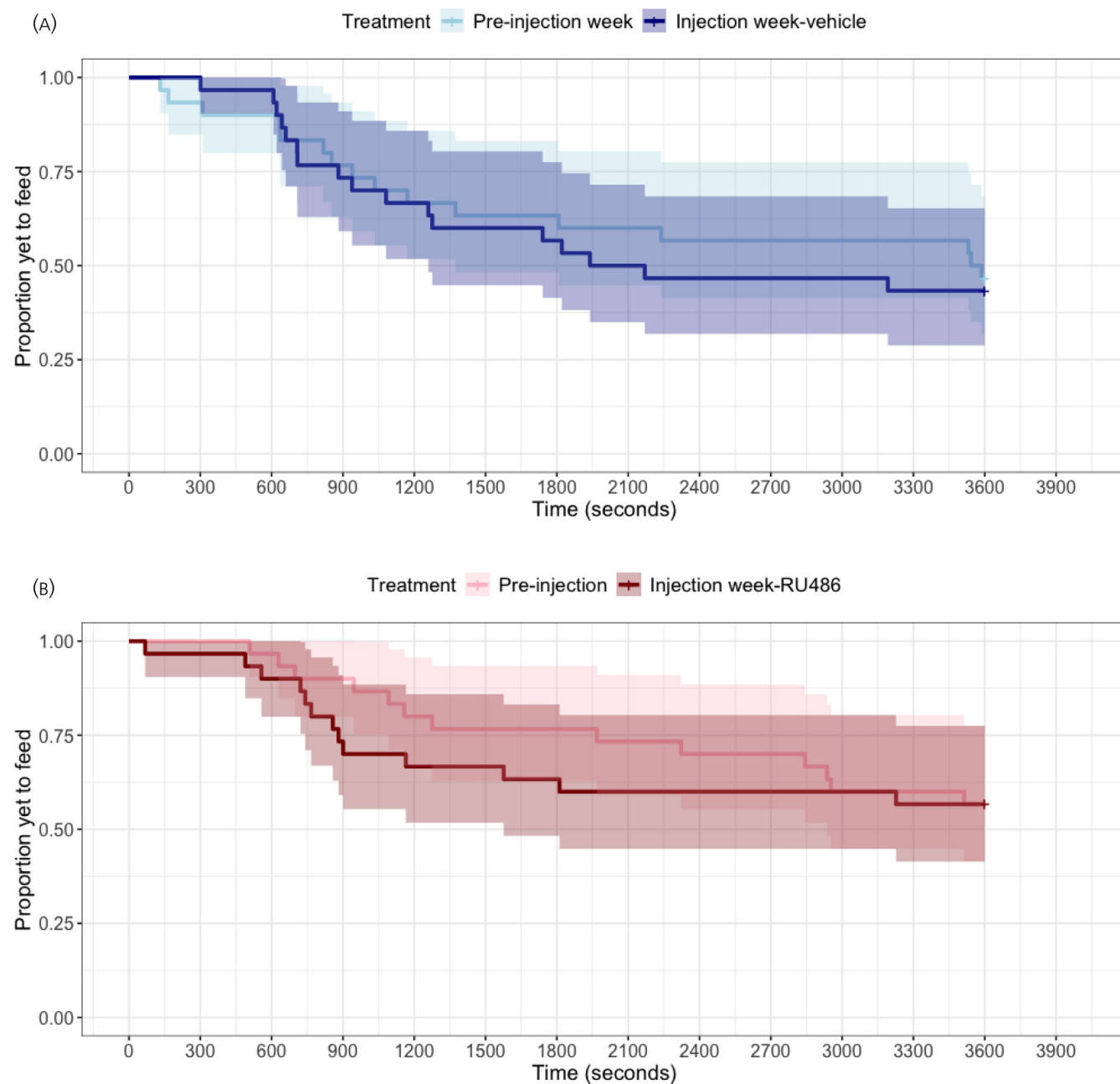


FIGURE 6 There was no effect of the GR antagonist RU486 on neophobia in house sparrows. Latency to feed during novel object trials for the pre-injection (transparent lines) and injection week (bold lines) for sparrows that received (A) a vehicle control ($n = 10$) or (B) RU486 ($n = 10$). Solid lines show the average latency to feed in the presence of a novel object ($n = 6$ trials/bird; 3 during the pre-injection week, 3 during the injection week). Shaded regions show 95% confidence intervals.

shorebirds that did not find effects of RU486 injections on corticosterone.^{32,38} In fact, we expected circulating corticosterone might increase as RU486 prevented GR binding in the brain and disrupted negative feedback. However, there is some evidence that RU486 can also act as an agonist in the songbird brain,⁷² which could have induced negative feedback upon binding and caused the reduced circulating corticosterone levels that we observed. Additionally, rodent studies have found a suppressive effect of repeated RU486 administration on corticosterone.⁷¹ This unexpected result led us to conduct

a follow-up study where we examined the time course of RU486 effects on corticosterone 3 h post-injection after 1, 3, and 5 days of injections. This study clarified that RU486-induced suppression of stress-induced corticosterone was rapid and long-lasting, while baseline corticosterone was not significantly impacted over this slightly shorter time course. A previous study in the lab found that the drug mitotane decreased neophobia behavior in house sparrows²⁴; mitotane reduces both baseline and stress-induced corticosterone within 36 h post-injection in house sparrows.²³ In this study, we saw that

RU486 reduced stress-induced corticosterone starting on the first day of injection, but only reduced baseline corticosterone on the sixth-day post-injection and did not affect neophobia behavior. These results suggest that the effects of mitotane on neophobia seen in the previous Kelly et al.²⁴ study were driven by the reductions of baseline corticosterone and MR binding, rather than acting via stress-induced corticosterone and GR binding.

Overall, our results suggest that GR does not influence neophobia behavior in wild-caught house sparrows. Understanding how baseline and acute concentrations of corticosterone affect neophobia is becoming increasingly important as wild populations not only experience widespread novelty in their environments but also novelty combined with increased anthropogenic stressors, including environmental toxicants,⁷³ chronic noise pollution,⁷⁴ and acidification from carbon emissions,⁷⁵ all of which have been shown to affect HPA activity. If animals with increased baseline corticosterone concentrations or increased MR expression also have increased neophobia, this could hinder their ability to take advantage of resources in novelty-rich environments and ultimately affect population persistence.

AUTHOR CONTRIBUTIONS

Marquise S. Henry: Conceptualization; methodology; investigation; writing – original draft; writing – review and editing; resources. **Melanie G. Kimball:** Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; resources. **Ella B. Cochran:** Investigation; writing – review and editing. **Blake A. Dusang:** Investigation; writing – review and editing. **William J. Frazier:** Investigation; writing – review and editing. **Keegan R. Stansberry:** Investigation; writing – review and editing. **Tosha R. Kelly:** Investigation; writing – review and editing. **Emily G. Stelling:** Investigation; writing – review and editing; resources. **Christine R. Lattin:** Conceptualization; methodology; investigation; writing – original draft; writing – review and editing; resources; supervision.

ACKNOWLEDGMENTS

We would like to thank private landowners for access to capture sites and the animal husbandry staff and veterinarians at LSU.

FUNDING INFORMATION

This research was funded by NSF IOS-2237423 and start-up funds from Louisiana State University to CRL. MGK was supported by a P.E.O. Scholar Award and a Dissertation Year Fellowship from Louisiana State University. MSH was supported by a Huel D. Perkins Doctoral Diversity Fellowship from Louisiana State University.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest involved in this research.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jne.70009>.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

All applicable institutional guidelines and guidelines for the use and care of animals were followed. Permissions for this research were granted through IACUC protocol #92-2023 and Louisiana State Scientific Collection permits WD-23-006 and WD-24-011.

ORCID

Marquise S. Henry  <https://orcid.org/0000-0002-6256-1423>

Melanie G. Kimball  <https://orcid.org/0000-0001-9638-0893>

Ella B. Cochran  <https://orcid.org/0009-0003-9794-6862>

Blake A. Dusang  <https://orcid.org/0009-0001-2124-1036>

Keegan R. Stansberry  <https://orcid.org/0000-0001-5467-4471>

Tosha R. Kelly  <https://orcid.org/0000-0001-5999-4159>

Christine R. Lattin  <https://orcid.org/0000-0003-4030-4212>

REFERENCES

- Ducatez S, Audet JN, Lefebvre L. Problem-solving and learning in Carib grackles: individuals show a consistent speed-accuracy trade-off. *Anim Cogn*. 2015;18(2):485-496. doi:10.1007/s10071-014-0817-1
- Martin JM, Bertram MG, Saaristo M, et al. Impact of the widespread pharmaceutical pollutant fluoxetine on behaviour and sperm traits in a freshwater fish. *Sci Total Environ*. 2019;650:1771-1778. doi:10.1016/j.scitotenv.2018.09.294
- Mazza V, Czipperack I, Eccard JA, Dammhahn M. Cross-context responses to novelty in rural and urban small mammals. *Front Ecol Evol*. 2021;9: 661971. doi:10.3389/fevo.2021.661971
- Batisteli AF, Sarmiento H, Aurélio Pizo M. Nest reuse by pale-breasted thrushes reduces the chance of cowbird parasitism and allows earlier initiation of breeding. *J Field Ornithol*. 2021;92(2):105-114. doi:10.1111/jof.12363
- Brown GE, Demers EEM, Goldman JA, Singh A, Chivers DP, Ferrari MCO. Unpredictable risk enhances induced neophobia in northern red-bellied dace. *Anim Behav*. 2020;168:121-127. doi:10.1016/j.anbehav.2020.08.012
- Miller R, Garcia-Pelegrin E, Danby E. Neophobia and innovation in critically endangered *Bali myna*, *Leucopsar rothschildi*. *BioRxiv*. 1-22. 2022. doi:10.1098/rsos.211781
- Szabo B, Damas-Moreira I, Whiting MJ. Can cognitive ability give invasive species the means to succeed? A review of the evidence. *Front Ecol Evol*. 2020;8:187. doi:10.3389/fevo.2020.00187
- Greggor AL, Thornton A, Clayton NS. Neophobia is not only avoidance: improving neophobia tests by combining cognition and ecology. *Curr Opin Behav Sci*. 2015;6:82-89. doi:10.1016/j.cobeha.2015.10.007
- Magory Cohen T, Kumar RS, Nair M, Hauber ME, Dor R. Innovation and decreased neophobia drive invasion success in a widespread avian invader. *Anim Behav*. 2020;163:61-72. doi:10.1016/j.anbehav.2020.02.012
- Romero LM. Physiological stress in ecology: lessons from biomedical research. *Trends Ecol Evol*. 2004;19(5):249-255. doi:10.1016/j.tree.2004.03.008
- Schoech SJ, Rensel MA, Heiss RS. Short- and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: a review. *Curr Zool*. 2011; 57(4):514-530. doi:10.1093/czoolo/57.4.514

12. Spencer KA, Verhulst S. Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (*Taeniopygia guttata*). *Horm Behav*. 2007;51(2):273-280. doi:[10.1016/j.yhbeh.2006.11.001](https://doi.org/10.1016/j.yhbeh.2006.11.001)
13. Spencer RL, Deak T. A users guide to HPA axis research. *Physiol Behav*. 2017;178:43-65. doi:[10.1016/j.physbeh.2016.11.014](https://doi.org/10.1016/j.physbeh.2016.11.014)
14. Breuner CW, Wingfield JC. Rapid behavioral response to corticosterone varies with photoperiod and dose. *Horm Behav*. 2000;37(1):23-30. doi:[10.1006/hbeh.1999.1554](https://doi.org/10.1006/hbeh.1999.1554)
15. Crespi EJ, Denver RJ. Ontogeny of corticotropin-releasing factor effects on locomotion and foraging in the Western spadefoot toad (*Spea hammondi*). *Horm Behav*. 2004;46(4):399-410. doi:[10.1016/j.yhbeh.2004.03.011](https://doi.org/10.1016/j.yhbeh.2004.03.011)
16. Herr MW, Graham SP, Langkilde T. Stressed snakes strike first: hormone levels and defensive behavior in free ranging cottonmouths (*Agkistrodon piscivorus*). *Gen Comp Endocrinol*. 2017;243:89-95. doi:[10.1016/j.ygcen.2016.11.003](https://doi.org/10.1016/j.ygcen.2016.11.003)
17. Baugh AT, Schaper SV, Hau M, Cockrem JF, de Goede P, van Oers K. Corticosterone responses differ between lines of great tits (*Parus major*) selected for divergent personalities. *Gen Comp Endocrinol*. 2012;175(3):488-494. doi:[10.1016/j.ygcen.2011.12.012](https://doi.org/10.1016/j.ygcen.2011.12.012)
18. Maren S, Patel K, Thompson RF, Mitchell D. Individual differences in emergence neophobia predict magnitude of perforant-path long-term potentiation (LTP) and plasma corticosterone levels in rats. *Psychobiology*. 1993;21(1):2-10. doi:[10.3758/BF03327121](https://doi.org/10.3758/BF03327121)
19. Prasher S, Thompson MJ, Evans JC, El-Nachef M, Bonier F, Morand-Ferron J. Innovative consumers: ecological, behavioral, and physiological predictors of responses to novel food. *Behav Ecol*. 2019;30(5):1216-1225. doi:[10.1093/beheco/arz067](https://doi.org/10.1093/beheco/arz067)
20. Cavigelli SA, McClintock MK. Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. *Proc Natl Acad Sci USA*. 2003;100(26):16131-16136. doi:[10.1073/pnas.2535721100](https://doi.org/10.1073/pnas.2535721100)
21. Romero LM, Dickens MJ, Cyr NE. The reactive scope model—a new model integrating homeostasis, allostasis, and stress. *Horm Behav*. 2009;55(3):375-389. doi:[10.1016/j.yhbeh.2008.12.009](https://doi.org/10.1016/j.yhbeh.2008.12.009)
22. de Bruijn R, Romero LM. Prior restraint stress inhibits habituation to novel objects in the European starlings (*Sturnus vulgaris*). *J Exp Zool A Ecol Integr Physiol*. 2020;333(2):88-95. doi:[10.1002/jez.2327](https://doi.org/10.1002/jez.2327)
23. Breuner CW, Jennings DH, Moore MC, Orchinik M. Pharmacological adrenalectomy with mitotane. *Gen Comp Endocrinol*. 2000;120(1):27-34. doi:[10.1006/gcen.2000.7537](https://doi.org/10.1006/gcen.2000.7537)
24. Kelly TR, Lynch KI, Couvillion KE, et al. A transient reduction in circulating corticosterone reduces object neophobia in male house sparrows. *Horm Behav*. 2022;137:105094. doi:[10.1016/j.yhbeh.2021.105094](https://doi.org/10.1016/j.yhbeh.2021.105094)
25. Lattin CR, Romero LM. Seasonal variation in corticosterone receptor binding in brain, hippocampus, and gonads in house sparrows (*Passer domesticus*). *Auk*. 2013;130(4):591-598. doi:[10.1525/auk.2013.13043](https://doi.org/10.1525/auk.2013.13043)
26. Lattin CR, Waldron-Francis K, Richardson JW, et al. Pharmacological characterization of intracellular glucocorticoid receptors in nine tissues from house sparrow (*Passer domesticus*). *Gen Comp Endocrinol*. 2012;159:214-220. doi:[10.1016/j.ygcen.2012.08.007](https://doi.org/10.1016/j.ygcen.2012.08.007)
27. Breuner CW, Orchinik M, Hahn TP, et al. Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am J Physiol Regul Integr Comp Physiol*. 2003;285(3):594-600. doi:[10.1152/ajpregu.00748.2002](https://doi.org/10.1152/ajpregu.00748.2002)
28. Senft RA, Meddle SL, Baugh AT. Distribution and abundance of glucocorticoid and mineralocorticoid receptors throughout the brain of the great tit (*Parus major*). *PLoS One*. 2016;11(2):1-18. doi:[10.1371/journal.pone.0148516](https://doi.org/10.1371/journal.pone.0148516)
29. Kalman BA, Spencer RL. Rapid corticosteroid-dependent regulation of mineralocorticoid receptor protein expression in rat brain. *Endocrinology*. 2002;143(11):4184-4195. doi:[10.1210/en.2002-220375](https://doi.org/10.1210/en.2002-220375)
30. Landys MM, Ramenofsky M, Wingfield JC. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol*. 2006;148(2):132-149. doi:[10.1016/j.ygcen.2006.02.013](https://doi.org/10.1016/j.ygcen.2006.02.013)
31. Reul JMHM, De Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*. 1985;117(6):2505-2511. doi:[10.1210/endo-117-6-2505](https://doi.org/10.1210/endo-117-6-2505)
32. Landys MM, Piersma T, Ramenofsky M, Wingfield JC. Role of the low-affinity glucocorticoid receptor in the regulation of behavior and energy metabolism in the migratory red knot *Calidris canutus islandica*. *Physiol Biochem Zool*. 2004a;77(4):658-668. doi:[10.1086/420942](https://doi.org/10.1086/420942)
33. Bókony V, Kulcsár A, Tóth Z, Líker A. Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). *PLoS One*. 2012;7(5):e36639. doi:[10.1371/journal.pone.0036639](https://doi.org/10.1371/journal.pone.0036639)
34. Kimball MG, Gautreaux EB, Couvillion KE, Kelly TR, Stansberry KR, Lattin CR. Novel objects alter immediate early gene expression globally for ZENK and regionally for c-Fos in neophobic and non-neophobic house sparrows. *Behav Brain Res*. 2022;428:113863. doi:[10.1016/j.bbr.2022.113863](https://doi.org/10.1016/j.bbr.2022.113863)
35. Martin LB II, Fitzgerald L. A taste for novelty in invading house sparrows, *Passer domesticus*. *Behav Ecol*. 2005;16(4):702-707. doi:[10.1093/beheco/ari044](https://doi.org/10.1093/beheco/ari044)
36. Kelly TR, Kimball MG, Stansberry KR, Lattin CR. No, you go first: phenotype and social context affect house sparrow neophobia. *Biol Lett*. 2020;16(9):20200286. doi:[10.1098/rsbl.2020.0286](https://doi.org/10.1098/rsbl.2020.0286)
37. Fair J, Paul E, Jones J, Bies L. *Guidelines to the Use of Wild Birds in Research*. Ornithological Council; 2023.
38. Landys MM, Ramenofsky M, Guglielmo CG, Wingfield JC. The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. *J Exp Biol*. 2004b;207(1):143-154. doi:[10.1242/jeb.00734](https://doi.org/10.1242/jeb.00734)
39. Eliezer N, Hurd CB, Moudgil VK. Immunologically distinct binding molecules for progesterone and RU38486 in the chick oviduct cytosol. *Biochim Biophys Acta Mol Cell Res*. 1987;929(1):34-39. doi:[10.1016/0167-4889\(87\)90238-2](https://doi.org/10.1016/0167-4889(87)90238-2)
40. Moudgil VK, Lombardo G, Hurd C, Eliezer N, Agarwal MK. Evidence for separate binding sites for progesterone and RU486 in the chick oviduct. *Biochim Biophys Acta Mol Cell Res*. 1986;889(2):192-199. doi:[10.1016/0167-4889\(86\)90104-7](https://doi.org/10.1016/0167-4889(86)90104-7)
41. Rich EL, Romero LM. Exposure to chronic stress downregulates corticosterone responses to acute stressors. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(6):R1628-R1636. doi:[10.1152/ajpregu.00484.2004](https://doi.org/10.1152/ajpregu.00484.2004)
42. Romero LM, Reed JM. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp Biochem Physiol A Mol Integr Physiol*. 2005;140(1):73-79. doi:[10.1016/j.cbpb.2004.11.004](https://doi.org/10.1016/j.cbpb.2004.11.004)
43. Romero LM, Wingfield JC. Tempest, poxes, and predators, and people: stress in wild animals and how they cope. *Oxford Series in Behavioral Neuroendocrinology*. Vol 1; 2016. Oxford University Press.
44. Wingfield JC, Vleck CM, Moore MC. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran desert. *J Exp Zool*. 1992;264(4):419-428. doi:[10.1002/jez.1402640407](https://doi.org/10.1002/jez.1402640407)
45. Krasowski MD, Drees D, Morris CS, Maakestad J, Blau JL, Ekins S. Cross-reactivity of steroid hormone immunoassays: clinical significance and two-dimensional molecular similarity prediction. *BMC Clin Pathol*. 2014;14:33. doi:[10.1186/1472-6890-14-33](https://doi.org/10.1186/1472-6890-14-33)
46. Kimball MG, Lattin CR. The “seven deadly sins” of neophobia experimental design. *Integr Comp Biol*. 2024;64(1):38-54. doi:[10.1093/icb/icad127](https://doi.org/10.1093/icb/icad127)
47. Kimball MG, Lattin CR. Exploration of a novel environment is not correlated with object neophobia in wild-caught house sparrows (*Passer domesticus*). *Behav Processes*. 2023;210:104913. doi:[10.1016/j.beproc.2023.104913](https://doi.org/10.1016/j.beproc.2023.104913)

48. Lattin CR, Kelly TR, Kelly MW, Johnson KM. Constitutive gene expression differs in three brain regions important for cognition in neophobic and non-neophobic house sparrows (*Passer domesticus*). *PLoS One*. 2022;17(5):e0267180. doi:[10.1371/journal.pone.0267180](https://doi.org/10.1371/journal.pone.0267180)
49. Krajcir KJ, Kelly TR, Kimball MG, et al. Eurasian tree sparrows are more food neophobic and habituate to novel objects more slowly than house sparrows. *Biol Invasions*. 2024;26(11):3677-3693. doi:[10.1007/s10530-024-03403-5](https://doi.org/10.1007/s10530-024-03403-5)
50. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2024.
51. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: tests in linear mixed effects models. *J Stat Soft*. 2017;82(13):1-26. doi:[10.18637/JSS.V082.I13](https://doi.org/10.18637/JSS.V082.I13)
52. Wickham H, Averick M, Bryan J, et al. Welcome to the Tidyverse. *J Open Source Softw*. 2019;4(43):1686. doi:[10.21105/joss.01686](https://doi.org/10.21105/joss.01686)
53. Peterson RA. Finding optimal normalizing transformations via best-Normalize. *R J*. 2021;13(1):310-329. doi:[10.32614/rj-2021-041](https://doi.org/10.32614/rj-2021-041)
54. Russell L, Bolker B, Buerkner P, et al. Estimated marginal means, aka least-squares means. *Am Stat*. 2024;34(4):1-101.
55. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag; 2016. doi:[10.18637/jss.v035.b01](https://doi.org/10.18637/jss.v035.b01)
56. Therneau TM. *coxme: Mixed Effects Cox Models*. R-Package Description, 1-14. 2024.
57. McLaughlin AL, Westneat DF. House sparrows exhibit individual differences in generalization when confronted with different novel stimuli. *Ethology*. 2023;129(8):369-379. doi:[10.1111/eth.13374](https://doi.org/10.1111/eth.13374)
58. Therneau TM. *survival: a package for survival analysis in R*. R package version 2.38. 2021. <https://CRAN.R-project.org/package=survival>
59. In J, Lee DK. Survival analysis: part II – applied clinical data analysis. *Korean J Anesthesiol*. 2019;72(5):441-457. doi:[10.4097/kja.19183](https://doi.org/10.4097/kja.19183)
60. Kuitunen I, Ponkilainen VT, Uimonen MM, Eskelinen A, Reito A. Testing the proportional hazards assumption in cox regression and dealing with possible non-proportionality in total joint arthroplasty research: methodological perspectives and review. *BMC Musculoskelet Disord*. 2021;22(1):1-7. doi:[10.1186/s12891-021-04379-2](https://doi.org/10.1186/s12891-021-04379-2)
61. Kassambara A, Kosinski M, Biecek P, Scheipl F. *Survminer: Drawing Survival Curves using “ggplot2”*. R package version 0.4.9. R Package Version 0.4.7. 2021.
62. Oitzl MS, Flutterm M, Ron de Kloet E. The effect of corticosterone on reactivity to spatial novelty is mediated by central Mineralocorticosteroid receptors. *Eur J Neurosci*. 1994;6(7):1072-1079. doi:[10.1111/j.1460-9568.1994.tb00604.x](https://doi.org/10.1111/j.1460-9568.1994.tb00604.x)
63. Vozella V, Cruz B, Natividad LA, et al. Glucocorticoid receptor antagonist mifepristone does not alter innate anxiety-like behavior in genetically-selected marchigian sardinian (MSP) rats. *Int J Mol Sci*. 2021;22(6):1-14. doi:[10.3390/ijms22063095](https://doi.org/10.3390/ijms22063095)
64. Fischer CP, Romero LM. The effects of daily mitotane or diazepam treatment on the formation of chronic stress symptoms in newly captured wild house sparrows. *Conserv Physiol*. 2020;8(1):coaa118. doi:[10.1093/conphys/coaa118](https://doi.org/10.1093/conphys/coaa118)
65. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*. 2000;21:55-89. doi:[10.1210/edrv.21.1.0389](https://doi.org/10.1210/edrv.21.1.0389)
66. Lustberg D, Tillage RP, Bai Y, Pruitt M, Liles LC, Weinshenker D. Noradrenergic circuits in the forebrain control affective responses to novelty. *Psychopharmacology (Berl)*. 2020;237(11):3337-3355. doi:[10.1007/s00213-020-05615-8](https://doi.org/10.1007/s00213-020-05615-8)
67. Steketee JD, Silverman PB, Swann AC. Noradrenergic receptor mechanisms in neophobia. *Psychopharmacology (Berl)*. 1992;106(1):136-142. doi:[10.1007/BF02253601](https://doi.org/10.1007/BF02253601)
68. Breuner CW, Orchinik M. Pharmacological characterization of intracellular, membrane, and plasma binding sites for corticosterone in house sparrows. *Gen Comp Endocrinol*. 2009;163(1):214-224. doi:[10.1016/j.ygcen.2009.01.027](https://doi.org/10.1016/j.ygcen.2009.01.027)
69. Van Eekelen JAM, Kiss JZ, Westphal HM, De Kloet ER. Immunocytochemical study on the intracellular localization of the type 2 glucocorticoid receptor in the rat brain. *Brain Res*. 1987;436(1):120-128. doi:[10.1016/0006-8993\(87\)91563-0](https://doi.org/10.1016/0006-8993(87)91563-0)
70. Spiga F, Knight DM, Droste SK, et al. Differential effect of glucocorticoid receptor antagonists on glucocorticoid receptor nuclear translocation and DNA binding. *J Psychopharmacol*. 2011;25(2):211-221. doi:[10.1177/0269881109348175](https://doi.org/10.1177/0269881109348175)
71. Dalm S, Karssen AM, Meijer OC, Belanoff JK, de Kloet ER. Resetting the stress system with a mifepristone challenge. *Cell Mol Neurobiol*. 2019;39(4):503-522. doi:[10.1007/s10571-018-0614-5](https://doi.org/10.1007/s10571-018-0614-5)
72. Katz A, Mirzaton A, Zhen Y, Schlinger BA. Sex differences in cell proliferation and glucocorticoid responsiveness in the zebra finch brain. *Eur J Neurosci*. 2008;28(1):99-106. doi:[10.1111/j.1460-9568.2008.06303.x](https://doi.org/10.1111/j.1460-9568.2008.06303.x)
73. Lattin CR, Ngai HM, Romero LM. Evaluating the stress response as a bioindicator of sub-lethal effects of crude oil exposure in wild house sparrows (*Passer domesticus*). *PLoS One*. 2014;9(7):e102106. doi:[10.1371/journal.pone.0102106](https://doi.org/10.1371/journal.pone.0102106)
74. Blickley JL, Word KR, Krakauer AH, et al. Experimental chronic noise is related to elevated fecal corticosteroid metabolites in lekking male greater sage-grouse (*Centrocercus urophasianus*). *PLoS One*. 2012;7(11):e50462. doi:[10.1371/journal.pone.0050462](https://doi.org/10.1371/journal.pone.0050462)
75. Chambers DL, Wojdak JM, Du P, Belden LK. Pond acidification may explain differences in corticosterone among salamander populations. *Physiol Biochem Zool*. 2013;86(2):224-232. doi:[10.1086/669917](https://doi.org/10.1086/669917)

How to cite this article: Henry MS, Kimball MG, Cochran EB, et al. A glucocorticoid receptor antagonist affects corticosterone but not neophobia in wild-caught house sparrows (*Passer domesticus*). *J Neuroendocrinol*. 2025;e70009. doi:[10.1111/jne.70009](https://doi.org/10.1111/jne.70009)