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A transient reduction in circulating corticosterone reduces object neophobia in male house sparrows

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ABSTRACT

Aversive reactions to novelty (or "neophobia") have been described in a wide variety of different animal species and can affect an individual's ability to exploit new resources and avoid potential dangers. However, despite its ecological importance, the proximate causes of neophobia are poorly understood. In this study, we tested the role of glucocorticoid hormones in neophobia in wild-caught house sparrows (*Passer domesticus*, $n = 11$ males) by giving an injection of the drug mitotane that reduced endogenous corticosterone for several days or a vehicle control, and then examined the latency to feed when the food dish was presented with or without a novel object in, on, or near the dish. Each sparrow was exposed to multiple novel object and control trials and received both vehicle control and mitotane treatments, with a week between treatments to allow the drug to wash out. As found previously, all novel objects significantly increased sparrows' latency to feed compared to no object present. Reducing corticosterone using mitotane significantly reduced the latency to feed in the presence of novel objects. In control trials without objects, mitotane had no significant effects on feeding time. Although we have shown that corticosterone affects neophobia, further studies using specific receptor agonists and antagonists will help clarify the neurobiological mechanisms involved and determine whether baseline or stress-induced corticosterone is driving this effect. These results suggest that increased glucocorticoids (e.g., due to human-induced stressors) could increase neophobia, affecting the ability of individuals to exploit novel resources, and, ultimately, to persist in human-altered environments.

1. Introduction

Aversive reactions to novelty (or "neophobia" (Barnett, 1958)) have been described in a wide variety of different animal species (Damas-Moreira et al., 2019; Gormally et al., 2018; Lau et al., 2021; Pinto et al., 2021). Because neophobia is often repeatable within individuals and across different contexts (Cavigelli and McClintock, 2003; Herborn et al., 2010; Morinay et al., 2019; Rasolofoniaina et al., 2021), it may constitute a key aspect of an animal's exploratory temperament (Réale et al., 2007), and as such, both constrain the ability of individuals to exploit new resources and environments and help them to avoid potential dangers. In fact, several studies have shown that neophobia may be particularly important in determining why some individuals, populations, and species are able to persist in human-altered landscapes while others are not (Candler and Bernal, 2015; Cohen et al., 2020; Greggor et al., 2016). However, despite its ecological importance, the proximate causes of neophobia are poorly understood.

One current hypothesis is that individuals demonstrating more behavioral signs of fear towards novelty may also experience stronger stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, which secretes glucocorticoid hormones both at baseline concentrations and in response to stressors (Cockrem, 2007; Koolhaas et al., 2010). Certainly, several studies have shown that exposure to novel objects or environments can in itself cause increased circulating glucocorticoids, suggesting that novelty may be perceived as 'stressful' (Apfelbeck and Raess, 2008; Baugh et al., 2017; Cavigelli et al., 2007; Richard et al., 2008) (but see Mettke-Hofmann et al., 2006). However, thus far, evidence to support links between individual variation in circulating glucocorticoids and neophobia is mixed, with some studies showing increased glucocorticoid concentrations in neophobic individuals (a higher stress-induced corticosterone response to restraint: Baugh et al., 2012; a higher and faster stress-induced corticosterone

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response to a novel arena: Cavigelli and McClintock, 2003), others finding decreased glucocorticoid concentrations in neophobic individuals (baseline corticosterone: Maren et al., 1993; Prasher et al., 2019), and some studies finding no relationship between glucocorticoids and neophobia (baseline corticosterone: Arnold et al., 2016; fecal glucocorticoid metabolites: Garamszegi et al., 2012).

These diverse findings might be partly because different studies have assessed different aspects of HPA function (e.g., baseline vs. stress-induced concentrations of glucocorticoids, which bind to different populations of receptors and have distinct effects (Landys et al., 2006; Lattin et al., 2012)) and sampled animals at different time points (e.g., pre- vs. post-novel object exposure). Further, most studies have correlated endogenous concentrations of glucocorticoids with the behavioral response to novelty rather than directly manipulating glucocorticoids and observing the effects on neophobia. Studies where researchers have administered exogeneous glucocorticoids suggest that glucocorticoid hormones may affect neophobia, albeit in complex ways. For example, European starlings (*Sturnus vulgaris*) given an injection of corticosterone (the primary avian glucocorticoid hormone) did not alter their neophobia behavior in response to novel objects compared to animals given a vehicle control, but there was a significant increase in approach latency towards some objects after a restraint stressor that elevated corticosterone (de Bruijn and Romero, 2020). Laboratory rats given a single acute injection of corticosterone 90 min prior to testing showed decreased neophobia in an open field test, whereas repeated corticosterone injections over a period of 25 days increased neophobia (Skorzewska et al., 2006). Further, a study administering corticosterone to nestling zebra finches (*Taeniopygia guttata*) showed that postnatal corticosterone exposure decreased object neophobia several weeks later in male, but not female, birds (Spencer and Verhulst, 2007). These results demonstrate that further experimental approaches are needed to better understand the relationship between glucocorticoid hormones and neophobia.

If elevated baseline or stress-induced glucocorticoid concentrations help mediate aversive responses to novelty, then reducing glucocorticoids should reduce neophobia. To test this hypothesis, we used the drug mitotane to temporarily lower endogenous corticosterone concentrations in wild-caught house sparrows (*Passer domesticus*) (Breuner et al., 2000) and tested neophobia in response to novel objects both after receiving a single dose of mitotane (the effects of which last several days) and after a vehicle control. Note that because mitotane reduces both baseline and stress-induced corticosterone, a mitotane effect on neophobia would not allow us to disentangle whether altered baseline or stress-induced corticosterone was responsible for the change in behavior. House sparrows display wide and repeatable individual variation in neophobia behavior in both the lab and the wild (Bokony et al., 2012; Ensminger et al., 2012; Fischer et al., 2020; Kelly et al., 2020; Martin and Fitzgerald, 2005), making them an excellent model to examine how neophobia may be impacted by experimental manipulation of glucocorticoids. We predicted that mitotane would reduce the latency of house sparrows to feed in the presence of a novel object but would not affect the latency to feed when novel objects were not present.

2. Methods

2.1. Subject capture and housing

We captured 11 male house sparrows in East Baton Rouge Parish, Louisiana, USA, using mist nets near bird feeders in July 2020. Sparrows were sexed and aged using plumage features (Lowther and Cink, 2006); all animals were adults. Although we only used males in this study, we have not observed any sex differences in neophobia in this species (e.g., Kelly et al., 2020). We individually housed sparrows in cages in a vivarium at Louisiana State University with unlimited access to mixed seeds, grit, a vitamin-rich food supplement (Mazuri Small Bird Diet), water, multiple perches, and a dish of sand for dustbathing (sand baths were removed before behavior trials). Sparrows were solo housed rather than group housed to avoid potential effects of social interactions on neophobia (Kelly et al., 2020). Day length in the lab corresponded to natural day length at the time of capture (13L:11D) and was maintained at this level for the duration of the experiment. Sparrows were visually but not acoustically isolated from one another to prevent observations of their neighbor's novel object trials. Sparrows received an aluminum band with a unique number and were held in captivity for 12 weeks before novel object trials began in November, during which time we conducted a pilot study to try to administer mitotane via diet. This pilot was not successful, so we administered mitotane via intramuscular injections as done previously (Breuner et al., 2000; Lattin et al., 2012). Sparrows were collected under a Louisiana Scientific Collecting Permit, and all procedures approved by the Louisiana State University Institutional Animal Care and Use Committee (project 70-2019). We used approved methods for bird capture, transport, and husbandry as specified in the Ornithological Council's Guidelines to the Use of Wild Birds in Research (Fair et al., 2010), and, at the project's completion, approved methods of euthanasia (isoflurane overdose) as specified in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Study design

This study took place over three weeks: the first week included five days of neophobia trials starting one day after a mitotane (or vehicle control) injection; week two served as a washout week; and the third week included another five days of neophobia trials in which the treatment (mitotane or vehicle control) each sparrow received was reversed. Thus, each sparrow served as its own control. Specifically, the day prior to neophobia trials (day 0 at 10:00), six house sparrows received a 100 μl injection of mitotane sonicated in peanut oil at a concentration of 90 mg/ml into the pectoralis muscle (dose: 350–450 mg/kg body weight) and five house sparrows received injections of 100 μl of peanut oil without mitotane (vehicle control). This method has been validated in house sparrows to reduce stress-induced corticosterone levels for at least five subsequent days and baseline corticosterone levels for at least three days (Breuner et al., 2000). Therefore, house sparrows were incapable of increasing circulating baseline corticosterone concentrations to normal levels over the subsequent three days (days 1–3) and stress-induced corticosterone over the subsequent five days (days 1–5) during neophobia trials, with stress-induced concentrations reaching a nadir around days 3–4 (Breuner et al., 2000). On days six and seven following neophobia trials, we restrained sparrows in clean, breathable cloth bags for 30 min and collected ~70 μl of blood from the alar vein using heparinized capillary tubes to assess whether sparrows were still unable to raise circulating corticosterone in response to an acute stressor (a standardized capture stress protocol) (Romero and Wingfield, 2016; Wingfield et al., 1992) 6 and 7 days post-injection, which, to our knowledge, has not been assessed. After an additional six days without experimental procedures (days 8–13; 13 days since the first injection), the experimental procedure was repeated, but sparrows received the opposite treatment, where sparrows that had previously received mitotane received a vehicle control injection, and vice-versa ($n = 5$ mitotane, $n = 6$ vehicle in week two; second injection on day 14; neophobia trials on days 15–19; blood sampling on days 20 and 21). Previous work has shown that 10 days after a single mitotane injection, house sparrows recover their ability to increase circulating concentrations of corticosterone in response to acute restraint (Breuner et al., 2000). Therefore, the sparrows that received the mitotane injection first had ample time to recover (13 days) before receiving the vehicle control.

2.3. Neophobia trials

Including both weeks of object exposures, we exposed house sparrows to a total of ten behavior trials, including four control trials (where no object was presented) and six novel object trials (two control and

three object trials each week, in a randomized order). Novel objects used were a blinking light, a white cover over part of the dish, yellow pipe cleaners, a purple plastic egg, a red-painted dish, and an opened blue cocktail umbrella. Objects were chosen to maximize the diversity of textures, colors, and shapes of novel objects. All objects were placed on, in, or immediately above the food dish and sparrows saw each object exactly once. The order of objects used was randomly determined for each individual sparrow. We have previously used all of these objects and shown that they significantly delay approach and feeding in house sparrows (Kelly et al., 2020).

The night before a trial, we removed food from cages 30 min before lights off. The next morning, 30 min after lights on, researchers entered the room, began a video recording of all cages (pole-mounted ZOSI Z18.5.Y.2 security cameras directed to a DVR), replaced food dishes according to individual treatments (control or object), and left for 1 h. Researchers were present in the room <4 min. Because sparrows do not feed after lights out (Lattin et al. in review), this only represents an additional 2 h of fasting at maximum if feeding does not occur during neophobia trials. This fasting period ensured that sparrows would be motivated to feed from the food dish. Upon re-entering the room, the video recording was stopped, and objects removed from food dishes for the rest of the day. Videos were scored blind to mitotane treatment for the time each sparrow took to eat from the food dish. All videos were scored by the same observer who re-watched a subset of videos one month later, blind to the original feeding time. Time to feed was scored exactly the same on the first and second watch, demonstrating that this measure is highly repeatable and reliable (see Appendix A, Supplementary data).

2.4. Corticosterone quantification

Day 6 and 7 post-injection stress-induced blood samples were stored on ice until centrifugation. We separated plasma from whole blood by spinning in a centrifuge at 5000 ×*g* for 8 min and stored at −80 °C until assay. We quantified circulating levels of corticosterone via Detect X Corticosterone Enzyme Immunoassay (EIA) Kits (Arbor Assays K014-H5, Ann Arbor, MI). We validated this kit for house sparrows by assessing parallelism in serial dilutions of house sparrow pooled plasma. Similar to Taff et al. (2019), we could not obtain parallelism when using the kit's dissociation reagent. We used double and triple ethyl-acetate extractions of corticosterone and found good parallelism (Stevenson and Purushothama, 2014) with 2-fold serially diluted samples from 1:5 to 1:40, though values from double-extracted samples were more variable and ~40% lower than triple-extracted samples. Therefore, we used triple ethyl-acetate extractions of 5 μl plasma samples as in Taff et al. (2019). Ethyl acetate was allowed to dry down overnight in a fume hood and corticosterone reconstituted in 125 μl of assay buffer for a final assay concentration of 1:25. We then proceeded with the EIA following the manufacturer's protocol. All samples were run in duplicate, with all Day 6 samples (mitotane and vehicle control samples for all sparrows) run on one plate and all Day 7 samples (mitotane and vehicle control samples for all sparrows) on a second plate. Extraction efficiency for this assay was determined by using stripped plasma samples spiked with a known amount of corticosterone and averaged 97%. Because extraction efficiency was high, we did not correct final plasma corticosterone values. Inter-plate variability was determined using the coefficient of variation of pooled plasma samples in different assays and averaged 14.8%. Intra-plate variability was determined using the coefficient of variation of duplicate samples and averaged 8.5%. The sensitivity of this assay was 20.9 pg/ml.

2.5. Statistical approach

We conducted all statistical analyses in R Studio version 4.0.2 (R Core Team, 2020). We used paired *t*-tests to assess whether mitotane treatment reduced the acute corticosterone response to restraint in sparrows on days 6 and 7 after injection (two tests) and used Cohen's d to calculate effect size estimates. There were two outliers in the day 6 corticosterone dataset: one from the vehicle control treatment and one from the mitotane treatment. Both values were over two standard deviations away from their respective group's mean (vehicle control mean = 41.7 ng/ml, vehicle control outlier = 78.8 ng/ml, 2.3 standard deviations away; mitotane mean = 29.8 ng/ml, mitotane outlier = 86.8 ng/ml, 2.4 standard deviations away) and were statistically significant outliers in their groups per a Grubb's test (vehicle control outlier: G = 2.3, $p = 0.03$; mitotane outlier: G = 2.4, $p = 0.02$). We therefore conducted a third paired *t*-test excluding the outliers, report results with ($n = 11$) and without ($n = 9$) these outliers and retain outliers in the figure.

Our behavior analysis included five Cox proportional hazard models, including three preliminary models to confirm that novel objects affected latency to feed from the food dish and to determine the appropriate covariates for the two models that tested for an effect of mitotane injection on neophobia behavior. All models used the 'coxme' package (Therneau, 2020) and model numbers in this paragraph correspond to the supplemental R code. Using a survival analysis approach (Kelly et al., 2020; Stankowich and Coss, 2007) avoids creating arbitrary threshold values when a subject does not perform the expected behavior during the allotted time period, which may bias alternative statistical approaches; i.e., giving sparrows a time of 3600 s if they do not feed during a 60 min trial. The first Cox proportional hazard model included sparrow ID as a random effect and a main effect of object type, in which "0" represented no object. The second and third Cox proportional hazard models were preliminary models to determine whether a main effect of (2) days post-injection or (3) trial number was a more appropriate covariate; both models included sparrow ID as a random effect. Specifically, we wanted to include either days post-injection or trial number as a covariate to control for repeated behavior trials and for variation in hormone levels due to the time course of mitotane. However, because the two were not independent, we could not include both effects in the model. The fourth and fifth Cox proportional hazard models tested our hypothesis and included sparrow ID as a random effect, treatment (mitotane or vehicle control), and trial number as fixed effects. Models four and five differed by trial type (i.e., one used data from control trials without an object, and the other used data from novel object trials) and included trials from both weeks. The purpose of the fourth model that only used data from no object control trials was to confirm that any effects of mitotane on behavior were specific to the presence of a novel object. The fifth model that used data from novel object trials assessed the effect of mitotane on responses to novel objects. We visualized the relationship between treatment (vehicle control or mitotane) and behavior using Kaplan-Meier survival curves (Kassambara et al., 2020). Two sparrows did not feed during any of the control trials where no object was present, and they were excluded from all behavior analyses (final $n = 9$ for Cox proportional hazard models); this response suggests an aversion to the entire testing procedure rather than neophobia per se. One final trial was excluded for a sparrow whose sand bath was not removed and ate a seed from the sand bath during a novel object trial. However, whether these trials (non-feeder trials and sand bath trial) are included or excluded does not affect the statistical significance of our behavior results.

Finally, we tested for a relationship between restraint-induced corticosterone and the latency to feed in the presence of a novel object to determine whether unmanipulated stress-induced corticosterone was correlated with neophobia, as in some previous work. We averaged day 6 and day 7 restraint-induced corticosterone from the week a sparrow received a vehicle control injection and regressed these values against the average latency of sparrows to feed in the presence of a novel object during that week (an average of three trials). We excluded the non-feeding sparrows for this analysis as well ($n = 9$) and estimated the effect size of this regression using Cohen's f^2. Data are presented as ± standard error of the mean (SEM). Raw data and R code are available in Appendix A, Supplementary data.

3. Results

Mitotane treatment significantly reduced sparrows' ability to increase circulating levels of corticosterone in response to restraint six days after a single injection compared to vehicle controls when outliers were removed from the analysis (Fig. 1, $t = 3.1$, df = 9, $p = 0.011$, d = 1.03; mitotane mean = 24.1 ± 4.9 ng/ml, vehicle control = 38.0 ± 3.5 ng/ml). Without outlier removal, the difference was not statistically significant ($t = 2.0$, df = 10, $p = 0.076$, d = 0.58; mitotane mean = 29.8 ± 7.2 ng/ml, vehicle control = 41.7 ± 4.9 ng/ml). On day 7 the lower restraint-induced corticosterone concentrations of mitotane-treated sparrows relative to vehicle controls was not statistically significant (Fig. 1, $t = 2.1$, df = 10, $p = 0.061$, d = 0.73; mitotane mean = 24.9 ± 4.4 ng/ml, vehicle control = 34.6 ± 3.6 ng/ml).

Novel objects presented on, in, or near the food dish significantly increased sparrows' latency to feed (all $p < 0.02$; Fig. 2). Days post-injection was not significantly associated with the latency to feed (object and control trials: z = 1.7, $p = 0.11$; mitotane only object trials: z = 0.54, $p = 0.59$); however, trial number was significantly associated with the latency to feed (object and control trials: z = 2.5, $p = 0.013$) and was therefore used as a covariate in models that investigated treatment effects. The interaction between treatment and trial number was not significant (object trials: z = −0.96, $p = 0.34$; control trials: z = −0.49, $p = 0.63$), so we removed this interaction from the final models. Whether or not this interaction was included in the final model did not affect the statistical significance of the main effects of treatment or trial.

Mitotane treatment significantly decreased the latency of sparrows to eat in the presence of a novel object (z = 3.7, $p = 0.0002$, hazard ratio (95% confidence interval) = 3.9 (1.9–7.9); trial number: z = 3.4, $p = 0.0007$; Fig. 3a) but did not affect the latency of sparrows to feed during control trials without objects (z = 0.4, $p = 0.7$, hazard ratio = 0.15 (0.5–2.5); trial number: z = 4.1, $p = 0.00004$; Fig. 3b). Hazard ratio calculations are reported in the supplementary material. During the trials that sparrows were injected with a vehicle control, restraint-induced corticosterone was not correlated with the average latency to feed in the presence of a novel object ($t_7 = -0.64$, $p = 0.54$, $R^2 = 0.05$, $f^2 = 0.06$).

4. Discussion

In this study, we found that a single injection of mitotane, a drug that lowers circulating baseline and stress-induced corticosterone for several days, was enough to significantly reduce neophobia in house sparrows. Specifically, sparrows were on average four times more likely to feed in the presence of a novel object after receiving a single injection of mitotane. These findings are consistent with the results of de Bruijn and Romero (2020), who showed that a restraint stressor that increased corticosterone also increased neophobia to some novel objects, and Skorzewska et al. (2006), who found that chronic corticosterone treatment increased neophobia in an open field test. The effect we observed was specific to novel objects, as mitotane-treated sparrows showed no change in feeding latency in control trials, where the food dish was

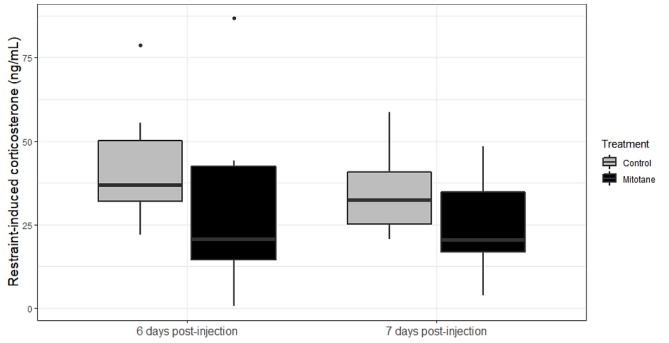

Fig. 1. Restraint-induced corticosterone concentrations (ng/ml) of house sparrows ($n = 11$) 6 and 7 days after receiving an intramuscular injection of mitotane (dose: 350–450 mg/kg body weight) suspended in peanut oil (mitotane; black) or peanut oil alone (vehicle control; grey). Box plots present the median (bold line), 25th and 75th percentiles (interquartile range [IQR]; box ends), and 1.5 × IQR (whiskers). Outliers (dots) are identified for day 6 post-injection. Corticosterone concentrations in mitotane-treated sparrows was significantly lower than vehicle controls on day 6 when outliers are excluded (outliers excluded: $p = 0.01$; outliers included: $p = 0.076$). This difference did not persist on day 7 ($p = 0.06$).

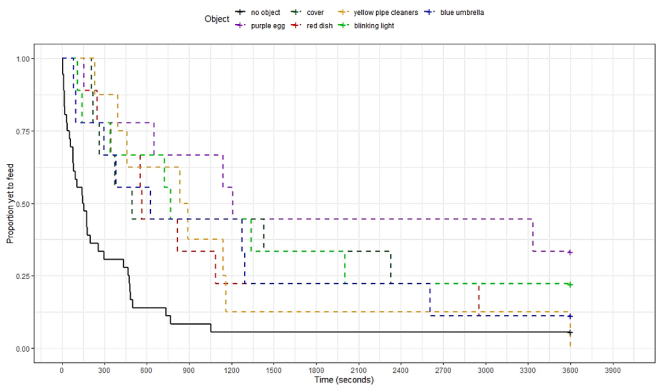

Fig. 2. Kaplan-Meier survival curves of house sparrow latency to feed during control trials where the normal food dish was presented (no object; solid black line, $n = 36$ trials from 9 males) compared to when a novel object was present (dashed coloured lines, $n = 53$ trials from 9 males). All objects significantly increased the latency of house sparrows to feed when compared to control trials when no object was present (all $p < 0.02$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

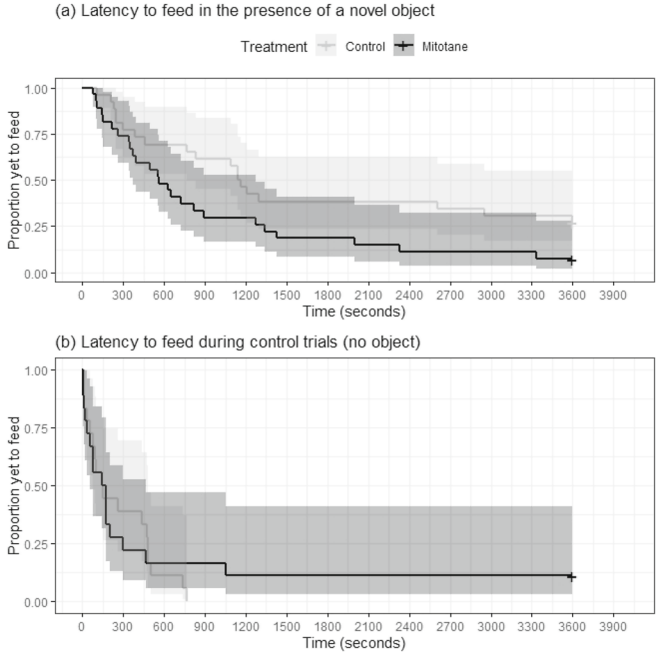

Fig. 3. Kaplan-Meier survival curves of male house sparrow ($n = 9$) feeding likelihood: a) in the presence of a novel object on, in, or near the food dish ($n = 26$ vehicle control trials and 27 mitotane trials) or b) during control trials when no object was present ($n = 18$ vehicle control and n = 18 mitotane trials). A mitotane injection (black) significantly reduced the latency to feed in the presence of a novel object compared to a vehicle control (grey), but did not affect the latency to feed when no novel object was present.

replaced without novel objects. In a previous study in house sparrows, mitotane was also found to reduce anxiety-related behaviors without affecting overall activity or feeding (Lattin et al., 2017).

There are two non-mutually exclusive possible explanations for the reduction in neophobia we observed. Mitotane is activated by an adrenal-specific cytochrome P450 enzyme, and in this active form, it blocks cytochrome P450-mediated reactions necessary for glucocorticoid production (Jonsson et al., 1994), affecting both baseline and stress-induced corticosterone concentrations. Therefore, mitotane should have reduced baseline corticosterone concentrations during behavior trials, and this background hormonal milieu may have partly determined the response to novel objects. Mitotane should also have attenuated any object- or human-induced increases in corticosterone, and this may have affected the response to novel objects as well. Past work has shown that novel object exposures can increase circulating glucocorticoids (Apfelbeck and Raess, 2008; Baugh et al., 2017; Richard et al., 2008), as can human presence (Nephew et al., 2003), which was necessary to replace food dishes in this study. Previous work has revealed that mitotane has a greater and longer-lasting impact on stress-induced than baseline corticosterone concentrations (Breuner et al., 2000; Lattin et al., 2017); therefore, we think it is more likely that these effects are mediated via attenuated stress-induced corticosterone. Reinforcing this, we did not find evidence for a decreasing effect of mitotane over time; effects of mitotane on baseline corticosterone should have been mostly gone by days 4 and 5 post-injection, but days post-injection was not related to latency to feed during object trials in mitotane-treated sparrows.

It should be noted, however, that we did not find a relationship between an individual sparrow's restraint-induced corticosterone concentrations and neophobia during novel object trials where mitotane was not administered. This could be because the response to restraint and the response to a novel object are not analogous. Studies in mammals (Romero et al., 1995) and birds (Nephew et al., 2003) have shown that different types of stressors elicit a range of corticosterone responses, and it is possible that the corticosterone responses to restraint and novel objects are uncorrelated. Although some previous work has found relationships between individual variation in stress-induced corticosterone (usually assessed using restraint) and neophobia (Arnold et al., 2016; Cavigelli and McClintock, 2003), including in house sparrows (Lendvai et al., 2011), other studies have failed to find such a link (Medina-Garcia et al., 2017). Further, the studies that have found a link between stress-induced corticosterone and neophobia do not always show the same relationship; that is, in some cases higher stress-induced corticosterone is associated with higher neophobia, but in other cases it is associated with lower neophobia. An additional consideration is that the HPA axis is a complex physiological system with many moving parts, and any manipulation affecting circulating corticosterone may also alter HPA mediators such as corticosterone binding globulin, enzymes that inactivate and regenerate corticosterone, the higher-affinity Type I (or mineralocorticoid receptor) primarily responsible for baseline corticosterone effects, or the lower-affinity Type II (or glucocorticoid receptor) that only shows significant binding at stress-induced corticosterone concentrations (Lattin et al., 2015; Malisch and Breuner, 2010; Rensel et al., 2018). A comprehensive examination of how different components of the HPA axis relate to neophobia – e.g., using specific agonists and antagonists for Type I or Type II receptors - would help clarify how glucocorticoids affect this behavior, and whether baseline or stress-induced corticosterone (or both) affect neophobia. Altogether, this study and previous work demonstrate that the relationship between corticosterone and neophobia is not a simple one.

Although we have shown that corticosterone affects neophobia, these effects may also be the result of corticosterone acting on other neurobiological systems that affect behavior rather than via corticosterone acting directly to change behavior. For example, many of the effects of glucocorticoids on the brain are mediated through rapid effects on the endocannabinoid system that alter glutamatergic, GABAergic, cholinergic, noradrenergic, and serotonergic neurotransmission (Balsevich et al., 2017; Popoli et al., 2011), with consequent impacts on behavior (Di et al., 2016; McReynolds et al., 2018). Further studies using specific receptor agonists and antagonists for endocannabinoid and other neuroendocrine receptors would help clarify the neurobiological mechanisms involved in corticosterone's effects on neophobia behavior in sparrows.

In conclusion, we present strong evidence of a role for glucocorticoids in mediating neophobia behavior in house sparrows. Wild animals are increasingly subject to human-induced stressors such as habitat degradation, chronic noise from human activities, and invasive predators, which may cause increased HPA activation and glucocorticoid secretion (Blickley et al., 2012; Chambers et al., 2013; Graham et al., 2012). Our results suggest that increased glucocorticoid exposure could increase neophobia, affecting the ability of individuals to exploit novel resources, and, ultimately, to persist in human-altered environments.

Declaration of competing interest

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2021.105094.

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