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RESEARCH ARTICLE

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Neophobia, but not perch hopping, is sensitive to long-term chronic stress intensity

Ursula K. Beattie[1] | Lily Mikolajczak[1] | Nina Fefferman[2] | L. Michael Romero[1]

[1]Department of Biology, Tufts University, Medford, Oregon, USA

[2]Department of Ecology and Evolutionary Biology, Department of Mathematics, University of Tennessee, Knoxville, Tennessee, USA

Correspondence
Ursula K. Beattie
Email: Ursula.beattie@tufts.edu

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Abstract

To further elucidate the role that wear-and-tear plays in the transition from acute to chronic stress, we manipulated the intensity and duration of applied chronic stress to determine if behavior would respond proportionately. We brought wild house sparrows into captivity and subjected them to high-stress, medium-stress, low-stress, or captivity-only. We varied the number of stressors per day and the duration of stress periods to vary wear-and-tear, and thus the potential to exhibit chronic stress symptoms. The behaviors we assessed were neophobia (the fear of the new; assessed via food approach latency) and perch hopping (activity). We predicted that our birds would show proportionate decreases in neophobia and activity throughout a long-term chronic stress paradigm. Our results indicate that neophobia is sensitive to the intensity of chronic stress, however, the birds became more neophobic, which was the opposite of what we expected. Conversely, perch hopping did not differ across treatment groups and is thus not sensitive to the intensity of chronic stress. Together, these data show that different behavioral measurements are impacted differently by chronic stress.

KEYWORDS

activity, behavior, chronic stress, house sparrow, neophobia, perch hopping, reactive scope

1 | INTRODUCTION

To date, the manner in which the adaptive acute stress response becomes pathological chronic stress is not well understood. The reactive scope model (Romero et al., 2009) posits that chronic stress could arise due to the accumulation of damage from many instances of acute stress (this accumulation termed wear-and-tear). According to a computational model of reactive scope, one understudied aspect of chronic stress that may help elucidate this transition is the intensity and duration of the stress. The current experiments are part of a larger project that aimed to manipulate the intensity and duration of chronic stress to manipulate wear-and-tear, and thus the manifestation of chronic stress symptoms (Beattie, Fefferman, et al., 2023; Beattie, Rosen, et al., 2023). We predicted that higher intensity chronic stress would have more physiological and behavioral effects on an animal.

Behavior is kept in a dynamic equilibrium through mediators such as hormones and neurotransmitters (Romero et al., 2009). These mediators can exist in four potential ranges: predictive homeostasis (levels to respond to daily and seasonal changes), reactive homeostasis (levels to respond to unpredictable changes/stressors), homeostatic overload (levels that are too high and cause damage themselves), and homeostatic failure (levels too low to sustain life). The transition from acute to chronic stress is akin to the transition from reactive homeostasis to homeostatic overload and reactive scope models wear-and-tear as a decrease of this threshold (Romero et al., 2009).

While the stress response is typically thought of as having two arms (the adrenomedullary response and the hypothalamic–pituitary–adrenal axis), the behavior response can be thought of as the third arm. These three arms are inherently linked, but they can also function independently (Fischer et al., 2016; Lendvai et al., 2011; Nephew, 2003). Regardless of physiological changes, an adjustment of behavior depending on the external environment can be the determining factor of whether or not an animal survives a stressor (Korte, 2001). Measuring neophobia, or the fear of the new, is one method of assessing these behaviors in an ecologically relevant manner (Greggor et al., 2015). Animals in the wild must balance the potential benefit of a new resource with the risk of predation or other adverse consequence (reviewed in Greenberg & Mettke-Hofmann, 2001). Neophobia can be affected by both chronic (e.g., Beattie et al., 2022; de Bruijn & Romero, 2021; Gormally & Romero, 2018; Gormally et al., 2018) and acute stress (e.g., de Bruijn & Romero 2019, 2021), as well as exogenous glucocorticoid manipulation (e.g., Kelly et al., 2022; Spencer & Verhulst, 2007), though the effects are not consistent. A decrease in neophobia (e.g., a faster approach to novel food) with chronic stress could indicate that an animal is more willing to take risks to obtain food (Crino et al., 2017; Martins et al., 2007), whereas an increase in neophobia (slower approach) could indicate a more anxious animal (Dickens et al., 2009).

In the present study, we altered a pre-existing method of inducing chronic stress (Rich & Romero, 2005) to create different intensity "levels" (Beattie, Fefferman, et al., 2023; Beattie, Rosen, et al., 2023) and monitored both neophobia and total activity (via perch hopping). If behavior is sensitive to the intensity of chronic stress, we would expect the most dramatic behavioral changes to be in higher intensity treatment groups.

2 | METHODS

2.1 | Experimental design

The current experiments were part of a larger project, the details of which are presented in Beattie, Rosen, et al. (2023) and repeated briefly here. We caught 40 adult or late-stage juvenile wild house sparrows (*Passer domesticus*) (17 M:23 F) with mist nets in Eastern Massachusetts in mid-June of 2021. Upon entering captivity, birds were doubly housed (M/F or F/F) in cages (45 cm × 37 cm × 33 cm), put on a light cycle of 12 L:12 D, and given ad libitum access to food (sunflower seeds and millet) and water for the acclimation period and throughout the duration of the experiment. They were given at least 3 weeks to acclimate to captivity before experiments began. During this time, the birds were undisturbed except for routine husbandry, such as food/water placement and cage changes.

After acclimation, the birds were randomly sorted into four treatment groups: captivity-only (5 M:5 F), low-stress (3 M:7 F), medium-stress (4 M:6 F), and high-stress (5 M:5 F). A true control group was not possible because housing wild animals in captivity is inherently stressful. The best achievable form of a control group was a captivity-only group, which experienced the stress of captivity, husbandry, and infrequent sampling, but no applied chronic stress. We compensated for the lack of a true control group by employing a repeated-measures design, where each bird served as its own control.

The other three treatment groups were subjected to an established chronic stress protocol (Cyr et al., 2007; Rich & Romero, 2005), in which acute psychological stressors are given multiple times per day at randomly chosen times. Stressors were 30 min long and included: cage rolling (rolling the cage racks around the room), cage tapping (tapping the cages with a pen), playing a radio in the bird room, human voice/presence in the bird room, restraint in a cloth bag, placing wind-up toys on the cage floor, running high-speed fans, and flashing colorful lights (always done during the scotophase). To vary the intensity and duration of chronic stress, we varied the number of stressors per day and the length of chronic stress bouts. All three chronic stress groups started the experiment with a 2-week bout of chronic stress (with four stressors per day) followed by a 2-week long break. The medium-stress group continued this paradigm five more times, for a total of six bouts of chronic stress. Each subsequent bout of chronic stress for the low and high-stress groups got shorter or longer by 2 days and the birds received fewer or more stressors per day, respectively. At the end of the 6-month repeating chronic stress period, all birds were given a prolonged recovery period of 6 weeks. A detailed timeline of the chronic stress regimen can be found in Figure 1. During periods of chronic stress, the stress birds were kept in a separate room than the captivity-only group. When certain groups needed more stressors, they were removed and stressors were applied in yet another room.

Throughout this long-term experiment, if any bird's weight dropped below 85% of its initial weight or showed signs of deteriorating health, the bird was removed from the experiment until it recovered. Some birds died for unknown reasons, however, a cox survival analysis (Cox, 1972; Therneau, 2022) showed no statistical significance of sample size across treatment groups ($z = 0.26$, $p = 0.79$; Beattie, Rosen, et al., 2023). A plot of the sample sizes at each timepoint can be found in Beattie, Rosen, et al. (2023). The age of the birds (aside from hatch-year or not) was not known, so it is possible that the birds that died were already toward the end of their lifespan.

All animals were collected under a Massachusetts state collection permit and all experiments were approved by the Tufts University Institutional Animal Care and Use Committee and were performed according to the guidelines for the use of wild birds in research (Fair et al., 2010).

2.2 | Behavior tests

Behavior tests were conducted at the start of the experiment and at the end of every bout of stress (Figure 1). We conducted behavior tests on the captivity-only group at the same timepoints as the medium-stress group. The present study was part of a larger project in which blood samples were taken to measure other stress-related metrics (Beattie, Fefferman, et al., 2023; Beattie, Rosen, et al., 2023) but behavior trials were never conducted at the same time as blood samples.

The night before a behavior trial, food was removed and cage liners were changed (to prevent birds from eating spilled food) 1 h before lights-off. Fasting the birds was necessary to motivate them to approach their food when an object was present. The next morning, food was replaced 10 min after lights-on (for a total fasting period of 13 h, 12 of which occurred during the scotophase). Birds had ad libitum access to water throughout the fasting period. During control trials, only food was added to the cages and during object trials, food dishes plus a novel

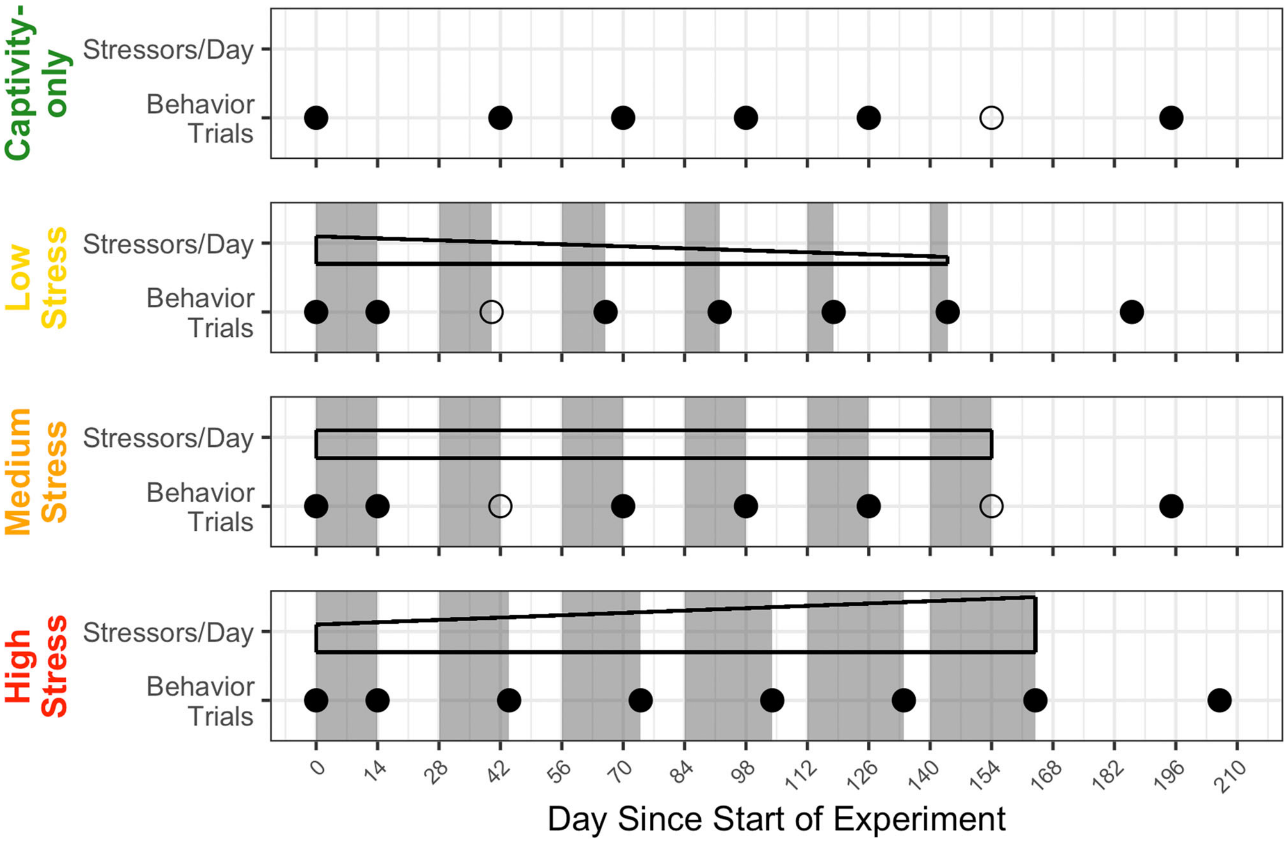

FIGURE 1 Timeline of chronic stress and samples taken. Panels correspond to the treatment group, with group names on the left. Gray rectangles indicate periods of applied chronic stress. Black-outlined shapes represent a decrease, maintenance, or increase in the numbers of stressors birds experience in a day. Dots indicate video samples for behavior analysis. Filled dots indicate timepoints with both control and novel object trials (on the following day), open dots indicate timepoints where control videos were lost, and thus only novel object trials exist.

object were added to the cages. We then used home security cameras to record the birds for 15 min at which point the cameras were turned off and the objects were removed. All novel objects had been previously validated to increase latency to approach the food dish (Beattie et al., 2022; Fischer et al., 2016) and consisted of an opened cocktail umbrella, a plastic easter egg, tulle, a Christmas ornament, a straw, a lei flower, a ribbon, a pipe cleaner bent into a spiral, an Altoids lid, Mardi Gras beads, and a regular food dish painted red. This array of objects was chosen to maximize differences in shape, color, texture, and size (Greggor et al., 2015). All objects were placed on, above, or in front of the food dish, but did not fully hide the food. Objects were randomized and no bird saw an object more than once, as habituation can occur within three exposures to the same novel object (de Bruijn & Romero, 2019). Control trials were always conducted the day before an object trial.

A blinded observer (L. M.) scored each video for approach latency and total hops. Other behaviors, like beak wiping, were occasionally seen, but never in sufficient numbers to analyze. Approach latency was scored as the time until a bird landed on its food dish. If a bird had not approached the food dish by 15 min, that datapoint was considered “censored” (14/234 instances for control trials, 172/261 instances for object trials; see statistics section). A hop was considered a significant movement throughout the cage. Hops were quantified during minutes 5–15 of the videos to avoid the confounding factor of food addition and experimenter presence (Gormally et al., 2018). To determine outliers, we averaged all values across the whole experiment and flagged datapoints that were ±3 standard deviations from the mean. Fourteen datapoints (out of 495 total) were removed for being higher than three standard deviations from the mean. Four control trial videos were lost: low stress and medium stress for postbout two and captivity-only and medium stress for postbout six (as indicated by open circles in Figure 1).

2.3 | Statistics

All statistics were performed in RStudio version 4.2.2 (RStudio Team, 2015). To analyze the neophobia data, we used a cox proportional hazard survival analysis, which statistically accounts for a subject not performing a certain behavior within the allotted time, which might have been arbitrarily chosen by the experimenter. In other words, if a bird had not approached the food dish by 15 min, we would have had to assign a ceiling value of 15 to run a linear mixed model. Using a survival analysis, we were able to “censor” the datapoints in which a bird did not approach the food (Kelly et al., 2020, 2022). Each trial was assigned a “status” in addition to the time to approach—1 means the bird successfully approached the dish, 0 means the bird did not approach the dish. We used the “coxme” function of the “coxme” package to run our survival analyses (Cox, 1972; Therneau, 2022). Our first model included treatment group (captivity-only, low stress, medium stress, and high stress), bout number (prebout 1, postbout 1–6), and trial (object trial or control trial) as fixed effects and bird ID as a random effect (to account for repeated measures). This statistical approach requires a “reference group” for comparisons, which was our captivity-only group for all models. Because trial was significant, we then split the data into two subsets (one for object trials and one for control trials) and ran a new model with treatment group and bout number as fixed effects and bird ID as a random effect, once for each subset. For the recovery data, we followed the same pipeline except without bout number (because there was only one recovery timepoint).

For the perch hopping data, we used linear mixed effect models (“lmer” function, lme4 package; Bates et al., 2015). This statistical approach is appropriate for hopping data because the data is number of hops within the timeframe, as opposed to time until first hop, which would have required a survival analysis. The first linear mixed effect model had treatment group, trial, and bout number as fixed effects and bird ID as a random effect. Then, we ran a Type III analysis of variance (ANOVA) (“ANOVA” function, car package (Fox & Weisberg, 2011) to test if the model was significant. Because trial was significant, we separated the hopping data into two subsets: one for object trial data and one for control trial data. We then ran a model with treatment group and bout number as fixed effects and bird ID as a random effect on each of those subsets and checked significance using a Type III ANOVA. For each step of the linear mixed effect model pipeline, we checked the assumption of homogeneity of variances using Levene's Test and by visually inspecting the residual plots for each model. Following Tukey's Ladder of Powers, we transformed the hopping data to the square root. For the recovery hopping data, we ran an initial model with group and trial as fixed effects and bird ID as a random effect. The medium group was excluded from this analysis as there were only three birds remaining. Trial was not significant in this model, so no further analyses were conducted.

3 | RESULTS

3.1 | Neophobia

Approach latency was significantly increased by the presence of a novel object ($z = -2.33$, $p = 0.02$; Figure 2 comparing top panels to bottom panels). This result provided statistical reasoning to separate the control trials and the novel object trials for the remainder of the analyses. Within control trials (Figure 2, top panels), there was no main effect of bout number ($z = 1.62$, $p = 0.11$; Figure 2a,b, top panels). The low-stress group approached significantly faster than the

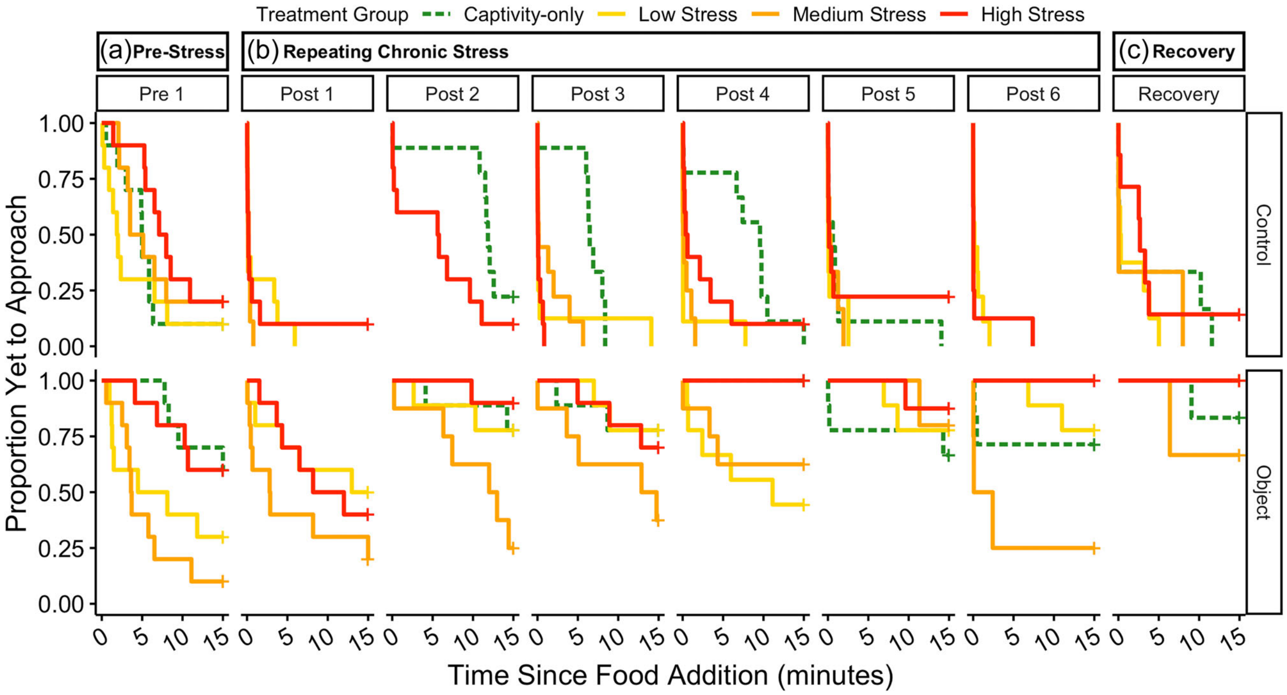

FIGURE 2 Survival curves of food approach likelihood before the stress experiment (a), throughout long-term chronic stress (b), and after 6 weeks of recovery (c). A proportion yet to approach of 1 means that 100% of the birds have not approached their food dish at a given time; as the proportions decrease with time (since food addition), more and more birds had approached their food. A plus sign (+) indicates birds that still had not approached their food by the end of the 15-min video. There was no change in approach likelihood through the chronic stress period (a,b) in the control trials (top panels), but chronic stress significantly increased latency to feed in the high-stress birds compared to the captivity-only birds (a, b, lower panels, red lines compared to green lines). There are no differences across treatment groups 6 weeks after the chronic stress period ended (c). Note that in the recovery timepoint, object trial (c, bottom) the high-stress group (red line) fully overlaps the low-stress group (yellow line, not visible).

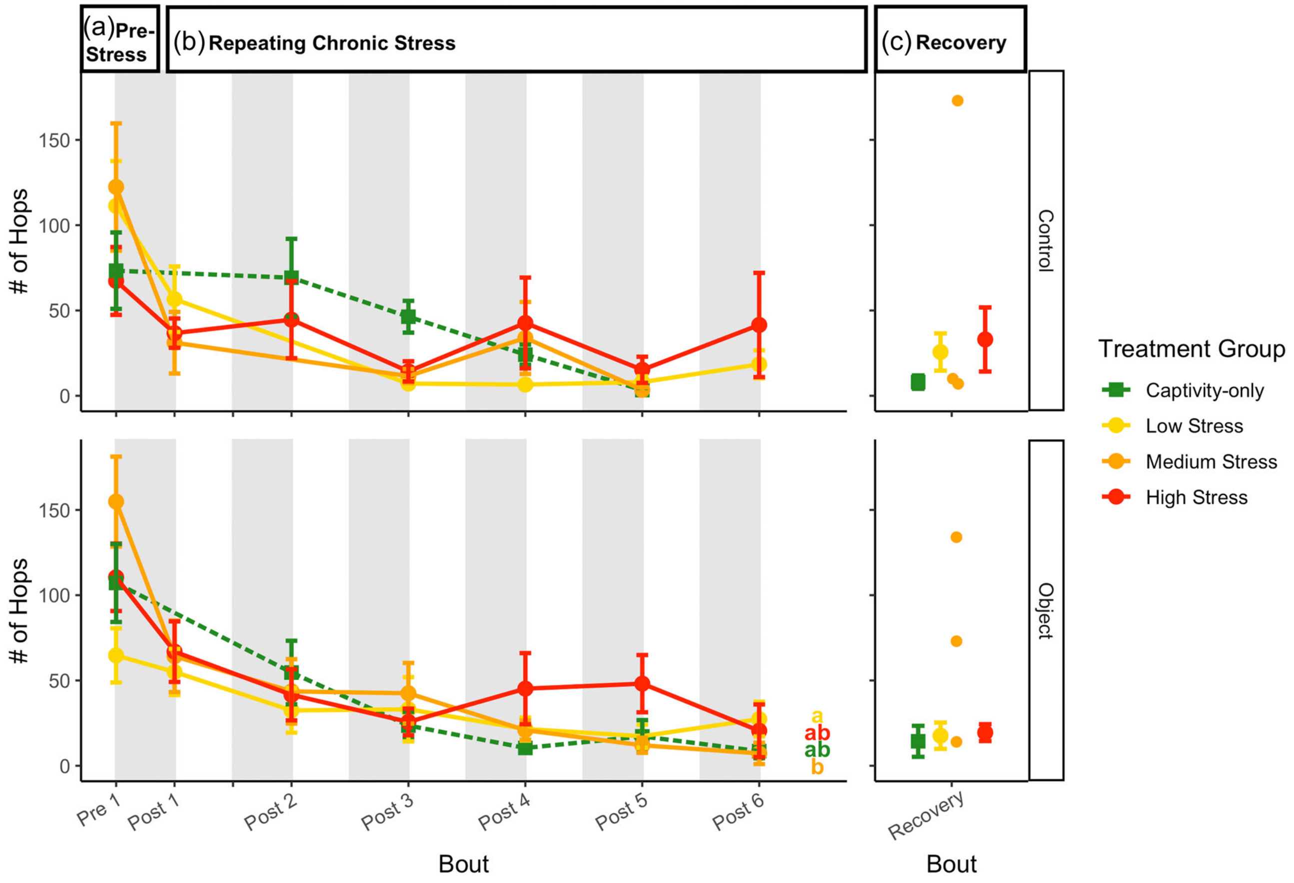

FIGURE 3 Total activity decreases through long-term chronic stress. There are no differences across treatment groups during control trials (top panels), but the low-stress group was less active than the medium-stress group during object trials, as indicated by letter significance markings (a, b, lower panel). This difference did not persist after 6 weeks of recovery (c, lower panel). There was no significant difference between trials during the recovery timepoint, but the data is graphed separately for comparison to the repeating chronic stress period. Data from the medium-stress group was excluded for the recovery timepoint statistics because only three birds remained, but data is graphed (as individual points) for completeness. Graphed values represent mean ± SE.

captivity-only group ($z = 2.39$, $p = 0.02$; Figure 2a,b, top panels, comparing the yellow lines to the green lines), but this did not change throughout the chronic stress experiment (interaction: $z = 0.32$, $p = 0.75$). The medium stress (main effect: $z = 1.66$, $p = 0.10$; interaction: $z = 0.77$, $p = 0.44$; Figure 2a,b, top panels, comparing the orange lines to the green lines) and high stress (main effect: $z = 0.75$, $p = 0.45$; interaction: $z = 0.73$, $p = 0.47$; Figure 2a,b, top panels, comparing the red lines to the green lines) groups did not approach differently than the captivity-only group.

When a novel object was present, there was no main effect of bout number ($z = -0.44$, $p = 0.66$; Figure 2a,b, bottom panels). The low-stress group did not approach differently than the captivity-only group (main effect: $z = 1.65$, $p = 0.10$; interaction: $z = -0.96$, $p = 0.34$; Figure 2a,b, bottom panels, comparing yellow to green lines). The medium-stress group approached their food faster than the captivity-only group ($z = 3.03$, $p < 0.005$; Figure 2a,b, bottom panels, comparing orange lines to green lines), but this did not change throughout the chronic stress experiment (interaction: $z = -1.19$, $p = 0.23$). Throughout the chronic stress period, the high-stress group (main effect: $z = 1.19$, $p = 0.23$; interaction: $z = -1.99$, $p = 0.04$; Figure 2a,b, bottom panels, comparing red lines to green lines) gradually approached their food slower than the captivity-only group as bout number progressed.

After 6 weeks of recovery, the presence of a novel object still increased approach latency ($z = -0.32$, $p < 0.005$, Figure 2c, comparing top panel to bottom panel). No treatment group approached differently than the captivity-only group during control trials (low stress: $z = 0.54$, $p = 0.59$; medium stress: $z = 0.40$, $p = 0.69$; high stress: $z = -0.36$, $p = 0.72$; Figure 3c, top panel, comparing yellow, orange, and red lines to green line) or novel object trials (low stress: $z = 0.00$, $p = 1.00$; medium stress: $z = 0.65$, $p = 0.52$; high stress: $z = 0.00$, $p = 1.00$; Figure 3c, bottom panel, comparing yellow, orange, and red lines to green line).

3.2 | Hopping

During the chronic stress period, there was a significant interaction between group and trial ($F_{3,350} = 3.19$, $p = 0.02$) and between bout and trial ($F_{6,349} = 2.17$, $p = 0.04$), which gave us statistical reasoning to split the control and object trials for further analyses (Figure 3a,b, comparing top panel to bottom panel). In control trials, hops significantly decrease with bout number ($F_{6,151} = 4.47$, $p < 0.001$; Figure 3a,b, top panel, comparing across bouts), but this effect is not dependent on group (main effect: $F_{3,179} = 1.69$, $p = 0.17$; group × bout interaction: $F_{13,153} = 1.70$, $p = 0.07$; Figure 3a,b, top panel, comparing across groups and bouts, respectively). In object trials, hops also decrease with bout ($F_{6,170} = 6.67$, $p < 0.001$; Figure 3a,b, bottom panel, comparing across bouts), but there is also a main effect of group ($F_{3,168} = 3.03$, $p = 0.03$) with post hoc tests showing that the medium-stress group hopped more than the low-stress group ($z = 2.96$, $p = 0.02$; Figure 3a,b, bottom panel, comparing the orange line to the yellow line). However, this effect seems to be driven by the medium-stress group having a higher number of hops than the low-stress group in the first timepoint as there is no significant interaction between group and bout ($F_{17,170} = 1.06$, $p = 0.40$; Figure 3a,b, bottom panel, comparing across bouts and groups).

During the recovery timepoint, there was no effect of trial or group on total hops (main effect of trial: $F_{1,19} = 0.19$, $p = 0.66$; main effect of group: $F_{3,32} = 1.69$, $p = 0.18$; group × trial interaction: $F_{3,19} = 0.58$, $p = 0.64$; Figure 3c comparing lines and panels). No further analyses were run but data from each trial were graphed separately (Figure 3c) for comparison to the chronic stress period (Figure 3a,b).

4 | DISCUSSION

The goal of this study was to manipulate wear-and-tear (Romero et al., 2009) caused by applied chronic stress and evaluate behavior as a downstream metric of the stress response (Wingfield, 2002; Wingfield et al., 1998). To determine if behavior is sensitive to the intensity and duration of chronic stress, we monitored neophobia and total activity (number of hops) in house sparrows that experienced only the stress of captivity, low stress, medium stress, or high stress. The present experiments were part of a larger project aimed at assessing if physiology and behavior are sensitive to the intensity of stress. Consequently, the most important comparisons of interest are across treatment groups. For example, immune function seems to exhibit more dramatic dysregulation in high-stress individuals (Beattie, Rosen, et al., 2023), but the effects on plasma metabolites are inconsistent (Beattie, Fefferman, et al., 2023).

4.1 | Neophobia

In the present study, no group approached food differently over time compared to the captivity-only group during control trials (no object present; Figure 2, top panels), but over time, only the high-stress group approached food slower than the captivity-only group during object trials (Figure 2, bottom panels). This was the opposite result than we expected, as chronic stress (<3 weeks) tends to either have no effect on neophobia (de Bruijn & Romero, 2021; Gormally & Romero, 2018) or makes birds less neophobic (a faster approach; Beattie et al., 2022; Gormally et al., 2018). However, two details should be noted: the birds in the present study experienced chronic stress for about six times as long as the previous studies to monitor neophobia through chronic stress (Beattie et al., 2022; de Bruijn & Romero, 2021; Gormally & Romero, 2018; Gormally et al., 2018) and the relationship between stress and behavior seems to be complicated. Even studies that isolate just the glucocorticoid arm of the stress response show conflicting data regarding its effects on behavior. A faster approach (less neophobia) can be induced by both a decrease (Kelly et al., 2022) or an increase (Spencer & Verhulst, 2007) in corticosterone. Similarly, increasing corticosterone can also decrease exploration (indicating more neophobia; Skórzewska et al., 2006) or have no effect (de Bruijn & Romero, 2019). This suggests that neophobic responses to chronic stress are highly sensitive to the context in which the chronic stress is applied.

The birds still exhibited a neophobic response to the foreign objects at the 6-week recovery timepoint, but surprisingly, there was not a difference across groups (Figure 2c). Visually, it appears as though the high-stress group did not recover to its prestress levels of neophobia, so the other three groups became more neophobic, to the level of the high-stress group. This result could indicate a latent effect of chronic stress on behavior that manifested earlier in the high-stress group and not until 6 weeks later in the captivity-only, low- and medium-stress groups. A previous study showed that through a single 3-week bout of chronic stress, birds initially became less neophobic but then gradually increased in neophobia throughout 2 weeks of recovery (Beattie et al., 2022). The present data suggest that this response might be accentuated with further bouts of chronic stress.

4.2 | Perch hopping

Perch hopping is used as a measure of activity (Gormally & Romero, 2020), which can increase with exogenous corticosterone (Astheimer et al., 1992; Breuner et al., 1998), but is dependent on both photoperiod and corticosterone concentration (Breuner & Wingfield, 2000). Throughout our study, all birds hopped less regardless of object presence and there were no differences in groups across time (Figure 3a,b). This data aligns with our observation that throughout the study, the birds became less agitated when we entered the bird rooms. In fact, near the end of the study, several birds did not move during the majority of the observation period. The present study also aligns with another study that showed a decrease in perch hops with chronic stress (Gormally et al., 2018), though sometimes this decrease is transient (Gormally & Romero, 2018), or not present at all (Beattie et al., 2022). The decrease in perch hopping over time that is seen in all groups could reflect a prolonged period of acclimation to captivity (longer than the 3 weeks before experiments began). Physiology and behavior can respond to the acclimation to captivity (Fischer et al., 2018) and chronic stress (Gormally & Romero, 2020; Gormally et al., 2019) on different timescales, so even though a bird could be considered hormonally acclimated after 2 weeks, its behavior could take much longer to acclimate.

Surprisingly, at the recovery timepoint (after about 8 months in captivity), the presence of a novel object did not affect hopping in our study (Figure 3c), though the birds continued to display neophobia as measured through food approach latency (Figure 2c), even in the captivity-only birds. Another study showed that wild house sparrows maintained neophobia as measured in both approach latency and perch hopping after being in captivity for 7 months (Beattie et al., 2022). The only major difference between these two studies is that the captivity-only birds in the present study had neophobia trials approximately once per month over 8 months, whereas the birds in the previous study did not have a neophobia trial until approximately 7 months of captivity (Beattie et al., 2022). Regardless of why the birds in the present study do not change their activity in response to a novel object during the recovery timepoint, this data makes the approach latency data even more intriguing. Overall, the birds were very inactive, but when food became available for the first time in 13 h, they rapidly approached, suggesting they were highly motivated to feed. During the object trials, however, the presence of a novel object was enough to prevent the birds from approaching, thus prolonging their fast.

5 | CONCLUSIONS

According to the reactive scope model, chronic stress can arise from the accumulation of damage (also known as wear-and-tear) from repeated stressors. In an effort to elucidate the transition into a chronic stress, we attempted to create varying levels of intensity of wear-and-tear to determine what, if any, aspects of physiology and behavior would show proportionate responses. Even within measures of behavior, two different responses were seen—food approach latency is sensitive to the intensity of chronic stress, while perch hopping (activity) is not.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ursula K. Beattie http://orcid.org/0000-0002-7131-3712
L. Michael Romero http://orcid.org/0000-0002-8854-8884

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