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Are novel objects perceived as stressful? The effect of novelty on heart rate

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HIGHLIGHTS

- We measured the heart rate response to novel objects in European starlings.
- Animals took longer to approach novel objects than their unaltered food dishes.
- Birds approached their dishes more quickly before any exposure to novelty.
- However, novelty did not cause an increase in the duration of the startle response.
- There were no correlations between behavior and heart rate metrics.

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ABSTRACT

Neophobia, or the fear of novel objects, is a behavior that is often found in wild animals. Neophobia appears to be related to the physiological stress response because individuals with higher glucocorticoid responses to stress often are more neophobic. The relationship between the heart rate response and novelty, however, has not been tested in a wild species. We implanted heart rate transmitters in captive European starlings (*Sturnus vulgaris*) to measure increases in heart rate as an index of the adrenomedullary stress response. Specifically, we measured heart rate in animals encountering novel objects on or near their food dishes using a system to display the novel objects while the experimenters remained outside the room, thereby minimizing the confounding effects of experimenter presence on heart rate. We analyzed three conditions: the period of adjustment to the experimental setup before any exposure to novelty, novel object trials, and no object controls (presented in a random order after 0–5 novel objects). Birds approached their food dishes faster during the adjustment period than during novel object trials. Although they demonstrated a behavioral aversion to novelty, the effect on heart rate was unexpected. Heart rate increased sharply when the food dishes were displayed. The duration of the startle response was longer during no object controls than during novel object exposure, the opposite of the anticipated result. There were no correlations between behavior and metrics of the heart rate response. Novel object exposure does not cause an increase in heart rate.

1. Introduction

Neophobia, the "fear of the new", is an ecologically relevant behavior characterized by an aversion to novelty, particularly novel objects. To an animal in the wild, a novel object could represent either a threat or a potential untapped resource. A neophobic animal may avoid the risks associated with novelty such as predation [6], but may not be able to gain access to new resources (reviewed by [18]). Neophobia is often assessed by placing novel objects on or near a food source [18]. The animals must weigh the potential threat of the object against their desire to approach the food. Neophobia has been measured this way in a number of wild bird species in captivity [1,5,20,24] and in the field [22,35]. In European starlings (*Sturnus vulgaris*) [14] and blue tits (*Cyanistes caeruleus*) [22] neophobic responses are equivalent in the wild and in captivity. Neophobic birds take much longer to approach a novel food dish or novel food type than they do their regular dish or food, but will rapidly habituate and no longer avoid novel objects as they encounter them multiple times [31]. Although fear of the new object may drive the behavioral responses, it is unclear whether the birds experience stress as they determine whether to approach an object they have never before encountered. The relationship between fear of novelty and stress is not fully understood.

The physiological stress response is a conserved reaction to a broad range of noxious situations that may be encountered by an animal, such as predator attacks, storms, disease, famine, etc. It consists of three arms. The adrenomedullary response begins in less than a second

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and consists of the increase in heart rate and mobilization of energy caused by the rapid release of catecholamine hormones (epinephrine and norepinephrine) from the adrenal medulla. The glucocorticoid response begins within minutes and can last for hours. It consists of the release of cortisol (in most mammals) or corticosterone (in birds) into the blood. Glucocorticoids have wide-ranging effects on many bodily functions, from energetics to immune function to reproduction to behavior (reviewed in [34]). The third branch of the stress response is the animal's adjustment of its behavior to appropriately confront the stressor. The three branches of the stress response are linked, but still maintain independent regulation from one another. For example, great tits (*Parus major*) show behavioral aversion to many stimuli, but they only increase glucocorticoid levels in response to some stimuli, i.e. a predator [8]. European starlings independently regulate their behavior, heart rate, and glucocorticoid levels in response to different types of stressful stimuli [10,28]. Neophobia resembles the behavioral arm of the stress response – it is a behavioral response to a stimulus which an animal might perceive as threatening.

The relationship between the glucocorticoid arm of the stress response and neophobia has been assessed in several bird species, with mixed results. The glucocorticoid response to a standardized stressor correlated with the behavioral response to novel objects in free-living Florida scrub jays (*Aphelocoma coerulescens*) [35], free-living house sparrows (*Passer domesticus*) [23], and great tits artificially selected for low neophobia [3]. In these studies, birds with strong glucocorticoid responses also had high fear and/or low exploration towards novel objects. However, in free-living collared flycatchers (*Ficedula albicollis*) [17] and zebra finches (*Taeniopygia guttata*) artificially selected for high and low corticosterone responses [25], there was no relationship between glucocorticoids and behavior towards novel objects. Novel objects cause either no or a very small glucocorticoid response in both European starlings [1] and Japanese quail (*Coturnix japonica*) [32] even when the birds demonstrate a strong behavioral response. In our previous experiments, an injection of corticosterone had no effect on neophobia in European starlings (unpublished data, D. Merullo and R. DeBruijn). In sum, it appears that there is a connection between neophobia and the glucocorticoid response, but it is still unclear whether neophobia activates the glucocorticoid arm of the stress response in birds. Furthermore, the time frame of a neophobic response and glucocorticoid release is not well matched.

In contrast, neophobic behavior and the heart rate response share a similar time frame that is much more rapid than glucocorticoid release, so perhaps neophobia activates the adrenomedullary arm of the stress response. The heart rate response to stress can be measured in European starlings using implantable heart rate transmitters [9,28]. Captive starlings show a robust startle response (i.e., a rapid increase in heart rate) within a fraction of a second of being exposed to various types of stressors [9,28]. In this study, we tested the hypothesis that novel objects will cause an increased heart rate in starlings. We measured heart rate in captive wild-caught European starlings during novel object exposure or exposure to the birds' normal food dish. Because the presence of a human causes a strong heart rate response in starlings [28], we created a system to expose hungry animals to either their familiar food dish or a dish modified with a novel object while the experimenter remained outside the room. We hid the food dish in an opaque black box that we lifted by pulling a string that ran outside the room where the birds were housed. When the black box was suddenly lifted, we anticipated that the animals would have a startle response (i.e., a sharp increase in heart rate). We hypothesized that heart rate would take longer to return to baseline when the animals were exposed to a novel object instead of their familiar dish. That is, the combined effect of startle and novelty would be greater than the startle effect alone. We further hypothesized that the animals would approach the food dish at approximately the time that their heart rate returned to baseline.

2. Materials and methods

2.1. Animals

Twelve European starlings were caught in March 2014 at a suburban dairy farm in eastern Massachusetts. They were housed in an outdoor aviary until late May 2014. They were then moved to an indoor animal facility and housed in individual cages on a 16L:8D light cycle. They were given at least four weeks to acclimate to the indoor facility. Heart rate could be measured on only four birds at a time. Two groups of four were tested in June–July; one group of four was tested in September. The birds from September had been used as controls in a previous experiment. Feather and blood samples had been taken from these birds one month or more before the beginning of this trial, but otherwise they were only exposed to standard caretaking. Just before experiments began, they were surgically implanted with heart rate transmitters and allowed to recover for several days (following [28]). Heart rate transmitters were purchased from Data Sciences International (St. Paul, MN). We used two different models, TA 10EA-F20 and TA 11ETA-F10. The transmitters send radio signals to a receiver attached to one side of each bird's cage. The data are then transferred to a computer equipped with Dataquest Advanced Research Technology Gold 4.0 software package, which records continuous ECG signals. The heart rate transmitters have a magnetic switch which could be turned on at the beginning of every experimental day. The birds' weights were monitored 1–2× weekly throughout the experiment. No significant weight changes were observed. We used a mix of males and females. There have been no previous differences found between the stress responses of captive males and females in this species [26–28]. All experiments complied with Association for Assessment of Laboratory Animal Care guidelines and were approved by the Tufts Institutional Animal Care and Use Committee.

2.2. Remote presentation of objects

Because we were looking for the heart rate response to novelty, we wanted to reduce the heart rate response to extraneous stimuli as much as possible. Therefore, we constructed a device to allow the animals to be exposed to novel objects without the presence of the researchers (Fig. 1). A black box constructed of foam core board was placed over the food dish. It was hinged onto the cage to prevent birds from entering from the top and was weighted with washers to prevent the birds from lifting it up. A string allowed researchers to pull the box up and display the food dish from outside the room. The behaviors of the animals were monitored using video cameras.

2.3. Neophobia trials

On the night before each experimental trial, the birds' food dishes were removed overnight to stimulate appetite and ensure the animals would be motivated to approach their food dishes in the morning. Opaque blinders were placed between the cages at the same time to prevent the birds from seeing one another's behavior and the objects the other birds were being exposed to. Starlings show more neophobia in isolation than when they are in contact with other starlings [1]. However, an individual bird's response to novelty can be affected by the behavior it has observed in others [16]. In the morning, birds were caught and their heart rate transmitters were switched on. At the same time, the opaque boxes were placed in the cages over the animals' food dishes (birds were restrained in a cloth bag for ~5 min while boxes were placed). The animals were then given 10 min for their heart rate to recover from handling stress. Baseline heart rate was then assessed for 20 min (beginning $t = -20$). At $t = 0$, the box was lifted, exposing birds to their food dish. Behavior and heart rate were monitored for 20 min. Heart rate transmitters were then switched off, boxes and novel objects removed from the cage, and the opaque dividers removed